

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
20 December 2001 (20.12.2001)

PCT

(10) International Publication Number
WO 01/96364 A2

(51) International Patent Classification⁷: **C07K 5/00**

Louise [GB/GB]; Dept. of Biochemistry, Imperial College, Exhibition Road, London SW7 2AZ (GB).

(21) International Application Number: PCT/GB01/02660

(74) Agents: **SILVESTON, Judith** et al.; Abel & Imray, 20 Red Lion Street, London WC1R 4PQ (GB).

(22) International Filing Date: 18 June 2001 (18.06.2001)

(25) Filing Language: English

(81) Designated States (*national*): JP, US.

(26) Publication Language: English

(84) Designated States (*regional*): European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR).

(30) Priority Data:
0014870.0 16 June 2000 (16.06.2000) GB

Published:

- without international search report and to be republished upon receipt of that report
- with sequence listing part of description published separately in electronic form and available upon request from the International Bureau

(71) Applicants (*for all designated States except US*): **IMPERIAL COLLEGE INNOVATIONS** [GB/GB]; 47 Prince's Gate, Exhibition Road, London SW7 2QA (GB). **KING'S COLLEGE LONDON** [GB/GB]; The Strand, London WC2R 2LS (GB).

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(72) Inventor; and

(75) Inventor/Applicant (*for US only*): **SAFFELL, Jane,**

(54) Title: PEPTIDES THAT STIMULATE CELL SURVIVAL AND AXON REGENERATION

(57) Abstract: Peptides which consist of or comprise the tetrameric peptide structural unit: Xaa-Xaa-Xaa-Xaa (SEQ.ID.NO.: 1) in which Xaa at position 1 represents Glu or Asp, Xaa at position 2 represents any amino acid, Xaa at position 3 represents any amino acid and Xaa at position 4 represents Glu or Asp, each of the meanings of Xaa being independent, and peptides which consist of or comprise the sequence PYSSA, particularly when in multimeric form, mimic the beneficial trophic and neuritogenic effects of FGF but lack the undesirable mitogenic effects. They are useful for the treatment of conditions for which FGF has been proposed, including treatment of neurodegenerative diseases, ischaemia, wound healing and stimulation of angiogenesis in cardiac muscle.



WO 01/96364 A2

PEPTIDES THAT STIMULATE CELL SURVIVAL AND AXON REGENERATION

FIELD OF THE INVENTION

This invention relates to neurodegenerative disease and nerve
5 damage, and more particularly to stimulators of axonal
regeneration and survival. The invention also relates to
wound healing and angiogenesis.

The need to stimulate neurite outgrowth, which is also called
10 axon regeneration, arises in the treatment of many diseases,
including peripheral neuropathies (for example, diabetic or
chemotherapy-induced), paralysis caused by spinal cord
injury, motor neurone disease, neurodegenerative diseases,
for example, multiple sclerosis, Alzheimer's disease, and
15 Parkinson's disease, and ischaemia, caused for example by
stroke.

Many agents can stimulate neurite outgrowth in vitro. Growth
factors, for example, nerve growth factor (NGF), fibroblast
20 growth factor (FGF), glial cell derived growth factor (GDNF),
brain derived growth factor (BDNF), and ciliary neurotrophic
factor (CNTF) have trophic (cell survival-promoting) and axon
regeneration effects, and there is much hope that they may be
useful in the treatment of damaged or diseased nervous
25 system. Some of the trophic factors have also been proposed
for use in promoting wound healing. Many of the factors have
been, are, or will be in clinical trial.

So far, however, the clinical improvements obtained with the
30 above-mentioned materials have been very disappointing,
mostly because of unwanted side-effects and short half life
of the proteins in blood. Many of the growth factors
mentioned above are mitogenic, and entail the risk that they
can stimulate tumour formation.

SUMMARY OF THE INVENTION

The present invention is based on the development of synthetic peptides, of much smaller molecular size than the prior materials, whose in vitro performance in stimulating axon regeneration and cell survival is comparable to that of the growth factors mentioned above. The novel peptides are (a) less prone to proteolysis and therefore should have a long half life in blood, (b) cheap and easy to produce in quantity, and (c) stable and easy to store without changes in activity between batches. These peptides do not stimulate mitogenesis and are therefore safer in this respect.

The peptides of the present invention appear *inter alia*, to mimic the beneficial trophic and neuritogenic effects of fibroblast growth factor (FGF) of high affinity receptor activation but to lack the undesirable mitogenic and apoptotic effects of FGF. The peptides of the invention may therefore be used in any of the situations where FGF is used, for example, they may be used for the treatment of any of the pathological conditions for which FGF has been proposed. Such conditions include conditions requiring stimulation of neurite outgrowth, conditions requiring stimulation of cell survival, for example, in neurodegenerative diseases, peripheral neuropathies, paralysis caused by spinal cord injuries, nerve damage caused by surgery or trauma, and ischaemia, for example caused by stroke, and in the treatment of multiple sclerosis (MS). The peptides may also be used to promote wound healing and to stimulate angiogenesis in cardiac muscle.

A further aspect of the invention relates to the inhibition of undesirable effects of FGF, for example, angiogenesis into tumours.

Accordingly, the present invention provides a pharmaceutical composition comprising a peptide of the invention in admixture with a pharmaceutically suitable carrier.

5

The invention also provides methods of treatment of a disease, disorder or pathological condition in a mammalian subject, especially a human subject, comprising administering to a subject in need of such treatment an amount of a peptide
10 of the invention effective for said treatment. The disease, disorder or pathological condition is especially as mentioned above, and in more detail below.

The invention also provides a peptide of the invention for
15 use as a medicament, for example, in the treatment of a disease, disorder or pathological condition as described above and below, and further provides the use of a peptide of the invention for the manufacture of a medicament for the treatment of a disease, disorder or pathological condition as
20 described above and below.

The peptides of the invention may be prepared by chemical synthesis or recombinantly. As an alternative to the use of a peptide of the invention, a nucleic acid molecule encoding
25 a peptide of the invention may be used in gene therapy, either in vivo or ex vivo.

BRIEF DESCRIPTION OF THE FIGURES

Figures 1a to 1k show the results of neurite outgrowth
30 assays. Figures 1a to 1h show the effect of various peptides of the invention on neurite outgrowth. Figures 1a to 1d compare the effect of peptides with soluble NCAM (Neuronal Cell Adhesion Molecule). The peptides used in Figures 1a to 1k are the following:

- Figure 1a: the 13-mer Ac-SIDRVEPYSSSTAQ-amide.
Figure 1b: the 10-mer Ac-DRVEPYSSSTA-amide.
Figure 1c: the tetramer Ac-DRVE-amide.
Figure 1d: the 6-mer Ac-PYSSSTA-amide.
- 5 Figure 1e: the 10-mers Ac-DRVEPYSSSTA-amide, Ac-ARVEPYSSSTA-amide, Ac-DRSEPYSSSTA-amide, Ac-ARSEPYSSSTA-amide.
Figure 1f: Peptide A(d), the multimeric peptide $[\{\text{Ac-DRVEPYSSSTA}\}_2\text{-K}]_2\text{-K-OH}$.
Figure 1g: the peptide Ac-DRVE-amide
- 10 Figure 1h: the peptide Ac-EGME-amide
Figure 1i shows the effect of FGF2 on cerebellar neurite outgrowth.
Figure 1j shows that 25 μ l of peptide Ac-DRVEPYSSSTA-amide inhibits neurite outgrowth stimulated by FGF2.
- 15 Figure 1k shows that cerebellar neurons from transgenic mice expressing dominant negative FGF receptors in neurons are not able to extend neurites in response to the peptide Ac-DRVEPYSSSTA-amide.
- 20 Figures 2a to 2i show the results of cell survival assays.
Figure 2a shows the effects of insulin (Figure 2a,i), Peptide A(d) (Figure 2a,ii) and FGF2 (Figure 2a,iii) on the survival of T3T cells in serum-free medium.
Figure 2b shows the effects of the multimeric peptide Ac-DRVEPYSSSTA[lys]₂[lys], i.e. peptide A(d), the "scrambled" multimeric peptide Ac-ADTRSVSEYP[lys]₂[lys], and the "mutated" multimeric peptide Ac-ARSEPYSSSTA[lys]₂[lys].
Figure 2c shows the effects of the peptides Ac-DRVE-amide and Ac-EGME-amide.
- 30 Figure 2d shows the effects of FGF2 and Peptide A(d) on the survival of L6 cells, which lack FGF2 receptors. Figure 2d compares the effects of DMEM, FCS, FGF2 at 0.1, 1 and 10 ng/ml, insulin at 0.5 and 5 μ g/ml, and Peptide A(d) at 100 and 400 μ g/ml.

Figure 2e,i shows the effect of FGF2 on 3T3 cell survival, demonstrating stimulation of cell survival at low concentrations and stimulation of apoptosis at high concentrations.

- 5 Figure 2e,ii shows the effects of inhibiting MAP kinase on cell survival in the presence of 10 ng/ml FGF2. The figure shows that the apoptotic effect of FGF2 can be reversed by supplementation with an inhibitor of MAP kinase signalling. Figure 2f is a Western blot showing that FCS, 1 and 10 ng/ml
- 10 FGF2 stimulate MAP kinase activation, while 0.1 ng/ml FGF2, the peptide Ac-DRVEPYSSSTA[lys]2[lys], and insulin do not. Figure 2g shows the effects of NGF, FGF2 and Peptide A(d) on survival of neurons.
- Figure 2h shows the effects of that Peptide A(d) on survival
- 15 of oligodendrocytes in vitro.
- Figure 2i shows cells cultured for three days in the presence of DMEM alone, or supplemented with insulin, FGF2 or Peptide A(d). Cells do not survive in the DMEM alone.
- Supplementation with insulin, low concentrations of FGF2 or
- 20 Peptide A(d) stimulate cell survival.
- Figure 3a shows the effects of Peptide A(d) on cell survival at two different cell plating densities, 5,500 cells/well and 11,000 cells/well, compared with that of FGF2 and insulin.
- Figure 3b shows the effects of insulin, Peptide A(d) and FGF2
- 25 on the incorporation of BrDU into cells, which is an indicator of mitogenesis.
- Figure 4 shows the structure of the multimeric, dendrimeric Peptide A(d).
- Figure 5 shows a generic multimer, denrimeric peptide having
- 30 four peptide units on a backbone structure of three lysine residues.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides peptides which consist of or comprise the essential tetrameric peptide structural unit:

5 Xaa-Xaa-Xaa-Xaa [SEQ.ID.NO.:1]

in which Xaa at position 1 represents Glu or Asp, Xaa at position 2 represents any amino acid, Xaa at position 3 represents any amino acid and Xaa at position 4 represents
10 Glu or Asp. Each of the meanings of Xaa is independent, that is to say, the residues at positions 1 and 4 may be the same as each other or may be different, and the residues at positions 2 and 3 may be the same as each other or different, and each may be the same as a residue at positions 1 and 4 or
15 may be different.

A peptide of the present invention may have an acyl group as substituent at the N-terminus and/or may be amidated at the carboxy terminus, for example, with an unsubstituted or
20 substituted amine group. An amino group may have one, two or three substituents, which may be the same or different, for example, selected from lower alkyl groups, in particular alkyl groups having up to four carbon atoms. An acyl group is preferably a lower acyl group, for example, having up to
25 four carbon atoms, especially an acetyl group.

A peptide of the invention may comprise the tetrameric structural unit set out above as part of a longer (extended) peptide molecule, some examples of which are given below. A
30 peptide of the present invention, in the form of a tetrameric structural unit as defined above or in the form of a longer peptide comprising such a unit may be present in a multimeric form, for example, as described below. The term "multimeric"

includes "dimeric". One or more peptides may be linked to a backbone structure, also called a scaffold molecule.

A peptide of the invention that comprises two cysteine
5 residues may be cyclised by formation of a disulphide bond between the two cysteine residues.

The tetrameric peptide structural unit may be any one of the following:

10 Glu - Xaa - Xaa - Glu [SEQ.ID.NO.:2]
 Glu - Xaa - Xaa - Asp [SEQ.ID.NO.:3]
 Asp - Xaa - Xaa - Glu [SEQ.ID.NO.:4]
 Asp - Xaa - Xaa - Asp [SEQ.ID.NO.:5]

in which each residue Xaa independently represents any amino
15 acid.

In the tetrameric peptide structural unit the amino acids at positions 2 and 3 may be natural or unnatural amino acids, for example they may be L-amino acids or D-amino acids, but
20 are preferably L-amino acids. Modified amino acids, for example, 4-hydroxyproline, gamma-carboxyglutamic acid, o-phosphoserine, o-phosphotyrosine and delta-hydroxylysine may be used.

25 It may be preferable to select the amino acids for positions 2 and 3 from arginine (R), glycine (G), methionine (M) and serine (S).

Examples of preferred tetrameric structural units are the
30 following: DRVE [SEQ.ID.NO.:6], EGME [SEQ.ID.NO.:7], EMGE [SEQ.ID.NO.:8], DRSE [SEQ.ID.NO.:9], DAVE [SEQ.ID.NO.:10], EVRD [SEQ.ID.NO.:11], EGGE [SEQ.ID.NO.:12].

As indicated above, a tetrameric peptide structural unit may be part of a longer peptide. Such a peptide of the invention may comprise, for example, up to 30 amino acids residues, for example, up to 25 amino acids residues, for example, up to 20
5 amino acids residues, for example, up to 15 amino acids residues, for example, 15, 14, 13, 12 , 11, 10, 9, 8, 7, 6, or 5 amino acids residues.

A peptide of the present invention may comprise the
10 tetrameric structural unit and the sequence PYSSTA [SEQ.ID.NO.: 13] or part of that sequence, preferably at the C-terminus of the tetrameric unit.

Examples of longer peptides of the present invention are the
15 following: DRVEPYSSTA [SEQ.ID.NO.:14], EGMEGM [SEQ.ID.NO.:15], DRSEPYSSTA [SEQ.ID.NO.:16], DAVEPYSSTA [SEQ.ID.NO.:17]. A peptide of SEQ.ID.NO.:14, 15, 16 or 17 may be extended at the N-terminus, at the C-terminus or at both termini. The N-terminus may be extended, for example, by an
20 isoleucine residue (I) or by serine-isoleucine (SI), and the C-terminus may be extended by a glutamine residue (Q). An example of such an extended peptide is SIDRVEPYSSTAQ [SEQ.ID.NO.:18].

25 Certain of the sequences set out above, in particular sequences consisting of or comprising the tetrameric structural unit DRVE [SEQ.ID.NO.:6] are derived from the first fibronectin type III repeat of NCAM, the neuronal cell adhesion molecule. The inventor has found that a soluble
30 NCAM-Fc chimeric molecule containing the first fibronectin type III domain (FNIII) stimulates survival of NIH T3T cells, whereas a soluble NCAM chimeric molecule lacking the first and second FNIII domains is unable to stimulate survival of NIH T3T cells. The NCAM molecule may have the sequence from

any suitable species. Preferably the sequence is that of a human NCAM molecule, including any human isoform of NCAM. The first fibronectin type III repeat of NCAM extends from proline at position 510 to glycine at position 600 of the human 140kd isoform (SEQ.ID.No. 21) (see databank entry GI:3334473), and the peptide of the invention may have any corresponding sequence of any other isoform or any other NCAM molecule.

10 Accordingly, the present invention provides a peptide that consists of or comprises the first fibronectin type III repeat of NCAM or any subsequence thereof, in particular any subsequence that comprises the tetrameric structural unit DRVE [SEQ.ID.NO.:6]. The peptide of the invention may be:

- 15 1) A peptide consisting of or comprising the first fibronectin type III repeat of NCAM;
- 2) A peptide consisting essentially of the first fibronectin type III repeat of NCAM or a peptide consisting essentially of or comprising a part of the first
- 20 fibronectin type III repeat of NCAM.
- 3) A peptide of 1) or 2) with other sequences from NCAM, for example one or more other NCAM domains.
- 4) A peptide of any one of 1), 2) or 3) as part of a fusion protein with non-NCAM sequences, e.g. an NCAM-Fc fusion
- 25 peptide.

The peptide may be an NCAM molecule lacking one or more domains providing that the first fibronectin type III repeat domain is present. The invention does not include the complete NCAM molecule or the use thereof.

30

The peptide of the invention may be a sequence having homology with one of sequences 1) to 4) above with the beneficial trophic and neuritogenic effects of FGF but

lacking the undesirable mitogenic effects of FGF, which beneficial properties are displayed by other peptides of the invention, for example of sequence ID.No.1. The homology may be at the amino acid level or at the nucleic acid level. At 5 the amino acid level, the sequence may have substitutions, additions or deletions. Substitutions are preferably conservative substitutions. The homology is preferably at least 80%, especially at least 90%, for example at least 95% at the amino acid level with a corresponding NCAM sequence, 10 for example the 140 kD isoform (SEQ.ID.No 21).

The peptide PYSSTA [SEQ.ID.NO.: 13] is also a subsequence of the first fibronectin type III repeat of NCAM. Accordingly, peptide PYSSTA, any peptide consisting of three or more, 15 especially four or five contiguous amino acids of peptide PYSSTA, and any peptide that comprises peptide PYSSTA or three or more, especially four or five contiguous amino acids of that peptide, is also part of the invention. In the sequence PYSSTA, there may be one or more substitutions, for 20 example one or two substitutions, especially conservative substitutions provided that the beneficial trophic and neuritogenic effects of FGF and the lack of the undesirable mitogenic effects of FGF (which beneficial effects are exhibited by the PYSSTA parent sequence) are retained. 25 Peptides that comprise the sequence DRVE and all or part of the sequence PYSTTA are particularly preferred.

Peptides tend to be more flexible than proteins because peptides lack the higher levels of organisation that tend to 30 maintain conformation in proteins. Cyclic peptides often have an advantage over linear peptides in that their cyclic structure is more rigid and hence their biological activity may be higher than that of the corresponding linear peptide. Peptides of the present invention may be linear or may be

cyclic. As described above, two or more cysteine residues may be provided in the peptide, thereby enabling formation of one or more disulphide bonds. The cysteine residues should not interrupt the tetrameric structural unit defined above, but may be at any other positions in the peptide molecule. It may be preferable to provide cysteine residues at or near the ends of the peptide molecule. For example, a cysteine residue may be provided at the N-terminus and at the C-terminus of any of the peptides set out above, for example, CDRVEPYSSSTAC [SEQ.ID.NO.:18].

Any peptide of the present invention, whether a tetrameric structural unit as such or a longer peptide comprising such a structural unit may be acylated at the amino terminus and/or may be amidated at the carboxy terminus, as described above. Amino acid side chains may also be modified, for example, at positions 2 and 3 of the tetrameric structural unit.

Any peptide of the invention may be reversed, that is to say, the N-terminal residue becomes the C-terminal residue and the sequence is written backwards. For example, the sequence DRVE [SEQ.ID.NO.:6] when reversed is EVRD [SEQ.ID.NO.:19], The invention includes all peptides in reversed form.

Any peptide of the present invention may be in multimeric form, that is to say, two or more peptides of the invention may be linked together via a backbone structure or scaffold molecule. Sometimes a smaller structure is called a linker group and a larger structure is called a backbone structure or scaffold molecule. However, the term "backbone structure" is used herein to denote the structure to which the peptides are linked, regardless of its size. The nature of the backbone structure is not critical, and many different different types of molecules may be used. One example of a

backbone structure is an oligolysine molecule, for example, having two or more lysine residues, for example two, three or more lysines. Two or more peptides of the invention, for example, two three or four peptides may be attached to the
5 lysine molecules, for example, to the amino side chains. A backbone structure, for example, an oligolysine molecule, may be linear or branched. A multimeric peptide of the invention on a branched backbone molecule may be referred to herein as a "dendrimeric" peptide. A generic, multimeric, dendrimeric
10 peptide having four peptide units linked to a backbone structure of three lysine residues is shown in Figure 5.

The following are examples of peptides of the invention.

15 1. Peptide A: Acetyl- DRVEPYSSSTA -amide

The peptide DRVEPYSSSTA is SEQ.ID.NO.:14

2. The multiple antigenic Peptide A(d):

20 [{Ac- DRVEPYSSSTA }₂ -K]₂ -K-OH

where Ac represents an acyl group, for example a lower acyl group, for example, having from 1 to 4 carbon atoms. This peptide is also referred to herein as Ac-

25 DRVEPYSSSTA[lys]2[lys]and as DRVEPYSSSTA[lys]2[lys].

3. Peptide B: Acetyl-DRVE-amide

DRVE is SEQ.ID.NO.:6

30 4. Peptide C: Acetyl-EGME-amide

EMGE is SEQ.ID.NO.:8

5. Peptide D: Acetyl- EGMEGM -amide

EGMEGM is SEQ.ID.NO.:15

6. Peptide E: Acetyl- DRSEPYSSSTA -amide
 DRSEPYSSSTA is SEQ.ID.NO.:16

5 7. Peptide F: Acetyl- DAVEPYSSSTA -amide
 DAVEPYSSSTA is SEQ.ID.NO.:17.

The invention also includes the reversed forms of the peptides of SEQ.ID.NOS.: 14, 6, 8, 15, 16 and 17, also of
 10 peptides A to E, for example, the reversed form of peptide SEQ.ID.NO.:14 is ATSSYPEVRD [SEQ.ID.NO.:19]. The reversed form of peptide A is Acetyl- ATSSYPEVRD -amide.

A peptide of the present invention, for example, one of the
 15 peptide molecules B to F above, or a reversed form of such a peptide, may form part of an extended structure by attachment to a suitable backbone structure, for example, as described above. For example, analogues of Peptide A(d) may be provided. Peptide A(d) has four units of peptide A
 20 (DRVEPYSSSTA) linked to a branched oligolysine backbone structure composed of three lysine residues, forming a "dendrimeric" peptide. Figures 4 and 5 show the structure of Peptide A(d).

25 Analogues of Peptide A(d) have four units of the respective acetylated peptide linked to the oligolysine backbone via one or more linker residues. For example, any of the following peptides may be linked to a branched backbone of three lysine residues: acetyl-DRVE-(X)_n , acetyl-EGME-(X)_n - , acetyl-EMGE-
 30 (X)_n - , acetyl-DRSE-(X)_n - , acetyl-DAVE-(X)_n - , acetyl-EVRD-(X)_n - , acetyl-EGGE-(X)_n - , acetyl-EGMEGM-(X)_n - , acetyl-DRSEPYSSSTA-(X)_n - or acetyl-DAVEPYSSSTA-(X)_n - , in which X is a linker residue or s direct bond and n is an integer of one or more, for example, 1,2 or 3. Suitable linkers are

known and include, for example, the dimer GS (glycine-serine) and multiples thereof, for example, GSGS and GSGSGS. An analogue may comprise a reversed form of a peptide.

- 5 Any of the other peptides of the present invention may be linked to a backbone structure to form a multimeric peptide. For example, a peptide that consists of or comprises the first fibronectin type III repeat of NCAM or any subsequence thereof, in particular any subsequence that comprises the
- 10 tetrameric structural unit DRVE [SEQ.ID.NO.:6] or, for example, the peptide PYSSTA [SEQ.ID.NO.: 13] or any peptide that consists or comprises all or part of that sequence. Again, reversed sequences may be used.
- 15 The peptides of the present invention stimulate axon regeneration, neurite outgrowth, and cell survival, including survival of neurons, oligodendrocytes and fibroblasts. As pointed out above, they demonstrate effects comparable to those obtained using various growth factors, for example,
- 20 nerve growth factor (NGF), fibroblast growth factor (FGF), glial cell derived growth factor (GDNF), brain derived growth factor (BDNF), and ciliary neurotrophic factor (CNTF), in particular FGF. As stated above, they appear to mimic the trophic and neuritogenic effects of FGF. These effects are
- 25 observed at low concentrations of FGF. Moreover, the peptides of the invention have an advantage over such growth factors, including FGF, in that they do not appear to have the disadvantageous mitogenic and apoptotic properties that generally accompany the useful growth stimulating properties
- 30 of the conventional growth factors. These effects are observed with FGF at high concentrations. The peptides appear to act by binding to and activating FGF receptors.

The need to stimulate neurite outgrowth arises in the treatment of many diseases, including peripheral neuropathies, for example, diabetic neuropathy or chemotherapy-induced neuropathy; paralysis caused by spinal
5 cord injury; neurodegenerative diseases, for example, motor neurone disease, multiple sclerosis, Alzheimer's disease, Huntingdon's disease, Parkinson's disease, PSP (progressive supranuclear palsy) and prion diseases; and ischaemia, caused for example by stroke; to stimulate angiogenesis in cardiac
10 muscle; and in restoration of nerve function after trauma or surgery.

The peptides of the present invention also stimulate cell survival. In this they seem to mimic the useful trophic and
15 neuritogenic effects of FGF without having the undesirable mitogenic and apoptotic effects. This is a general effect on cell survival, which is observed for example, in neurons, in oligodendrocytes, and in fibroblasts. The ability of a peptide of the present invention to stimulate cell survival,
20 in particular survival of neurons, enhances its effect on axon regeneration.

Oligodendrocytes are involved in the myelination of nerve cells. Demyelination is a key factor in multiple sclerosis
25 (MS). The ability of the peptides of the present invention to stimulate the survival of oligodendrocytes makes them useful in the treatment of MS.

Fibroblasts are involved in wound healing, so the ability to
30 stimulate fibroblast survival is important in promoting wound healing. Wounds include those caused, for example, by trauma, by surgery, by burns, for example, thermal burns, chemical burns and radiation burns, and also other radiation damage, for example, caused by radiotherapy.

The observations of the effects of the peptides of the present invention on cell growth and survival indicates that they appear *inter alia*, to mimic the beneficial trophic and neuritogenic effects of fibroblast growth factor (FGF) of high affinity receptor activation but to lack the undesirable mitogenic and apoptotic effects of FGF. The peptides of the invention may therefore be used in any of the situations where therapeutic use FGF has been proposed, or would be proposed were it not for the adverse side effects.

Accordingly, the present invention provides a method of stimulating neurite outgrowth in a mammalian subject, especially a human subject, comprising administering to a subject in need of such treatment an amount of a peptide of the invention effective for neurite outgrowth promotion.

The present invention also provides a method of stimulating cell survival in a mammalian subject, especially a human subject, comprising administering to a subject in need of such treatment an amount of a peptide of the invention effective for said cell survival promotion.

The method may be used, for example, for the stimulation of the survival of any cells whose survival is stimulated by FGF. For example, the method may be used to stimulate the survival of cells selected from neurones, oligodendrocytes and fibroblasts.

The present invention also provides a method of treatment of a neurodegenerative disease in a mammalian subject, especially a human subject, comprising administering to a subject in need of such treatment an amount of a peptide of the invention effective for said treatment.

A neurodegenerative disease is, for example, motor neurone disease, multiple sclerosis, Alzheimer's disease, Parkinson's disease, Huntingdon's disease, PSP (progressive supranuclear palsy) or a prion disease.

The present invention also provides a method of treatment of a peripheral neuropathy in a mammalian subject, especially a human subject, comprising administering to a subject in need of such treatment an amount of a peptide of the invention effective for said treatment.

A peripheral neuropathy is, for example, diabetic neuropathy or chemotherapy-induced neuropathy.

The present invention also provides a method of treatment for stimulating or restoring nerve function after trauma or surgery in a mammalian subject, especially a human subject, comprising administering to a subject in need of such treatment an amount of a peptide of the invention effective for said treatment.

The nerve function to be stimulated or restored may be local nerve function. This method of the invention may also be used in the treatment of paralysis caused by spinal cord injuries.

The present invention also provides a method of stimulating angiogenesis in cardiac muscle in a mammalian subject, especially a human subject, comprising administering to a subject in need of such treatment an amount of a peptide of the invention effective for said treatment.

The present invention also provides a method of treatment of ischaemia in a mammalian subject, especially a human subject, for example, ischaemia caused by a stroke, comprising administering to a subject in need of such treatment an
5 amount of a peptide of the invention effective for said treatment.

In a further aspect, a peptide of the present invention may be used to inhibit an undesirable effect of FGF, for example,
10 angiogenesis into tumours. Tumours require blood and secrete factors including FGF to stimulate the growth of blood vessels into themselves. A peptide of the present invention maybe able to block FGF binding to its receptor without itself stimulating the receptor, thus inhibiting the
15 angiogenic effect of FGF.

The present invention thus provides a method of inhibiting or reducing angiogenesis in a tumour in a mammalian subject, especially a human subject, comprising administering to a
20 subject in need of such treatment an amount of a peptide of the invention effective for said inhibition.

The present invention also provides a peptide of the invention for use as a medicament, especially for any of the
25 methods of treatment described above.

The invention further provides the use of a peptide of the invention for the manufacture of a medicament, particularly for any of the methods of treatment described above.
30

A peptide of the invention should be administered by a route appropriate for the condition to be treated and for the treatment desired. Such routes include intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous,

intranasal, epidural, and oral routes. The peptides may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings for example, oral mucosa, rectal and
5 intestinal mucosa, and may be administered together with other biologically active agents. Administration can be systemic or local.

The present invention provides a pharmaceutical composition
10 comprising a therapeutically effective amount of peptide of the invention and a pharmaceutically suitable carrier. The term "carrier" includes diluents, adjuvants, excipients, and vehicles with which the peptide is administered. The term carrier also includes wetting and emulsifying agents, and pH
15 buffering agents which, if present, are generally present in minor amounts. Compositions of the invention can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. Examples of suitable pharmaceutical carriers are
20 described in "Remington's Pharmaceutical Sciences" by E. W. Martin. Such compositions will contain a therapeutically effective amount of the peptide, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The
25 formulation should suit the mode of administration. The journal "Advanced Drug Delivery Reviews" is a useful source of information on new and more sophisticated pharmaceutical formulations.

30 In one embodiment, the pharmaceutical composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic

aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anaesthetic such as lignocaine to ease pain at the site of the injection. For administration to promote wound healing, it is preferable to
5 provide a formulation suitable for topical application, for example, a cream or ointment. Impregnated dressings and sprays are particularly useful.

For treatment of neurodegenerative diseases that involve
10 brain degeneration, it is preferable to use a formulation suitable for delivery of the peptide across the blood-brain barrier. Examples of suitable formulation are given in "Delivery of peptides, and proteins through the blood-brain barrier" Bickel U et al. Advanced Drug Delivery Reviews 46,
15 247-279.

The amount of the peptide of the invention which will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or
20 condition, and can be determined by standard clinical techniques. In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges. However, suitable dosage ranges for intravenous administration are generally about 20-500 micrograms of active compound per
25 kilogram body weight. Suitable dosage ranges for intranasal administration are generally about 0.01 pg/kg body weight to 1 mg/kg body weight. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal test systems.

30

A peptide of the present invention may be produced by conventional methods of chemical synthesis, for example, using the Merrifield technique. Such methods are well known and well documented.

Alternatively, a peptide, particularly a longer peptide of the invention, may be produced recombinantly. A nucleic acid encoding the peptide may be synthesised chemically or the
5 domain of the NCAM gene comprising the first fibronectin type III repeat may be used. The sequence of the human 140kd isoform of NCAM is available under the database reference GI:3334473. The first fibronectin type III repeat in that sequence comprises the amino acids from proline at position
10 510 to glycine at position 600. B28 cells stably infected with wild-type human L1 NCAM cDNA have been described (Lin et al 1996 J Cell Biochem 63:463-477 & Lin et al 1996 Mol Biol Cell 7:1977-1993, Zisch et al, 1997 J Neurosci Res 47:695-665 & Dahlin-Huppe 1997 Mol Cell Neurosci 9:144-156).

15

For recombinant expression of a peptide of the invention the nucleic acid encoding the peptide can be inserted into an appropriate expression vector, i.e., a vector that contains the necessary elements for the transcription and translation
20 of the inserted coding sequence. In a preferred embodiment, the regulatory elements for example, promotor, are heterologous i.e., not the native gene promotor. Promoters which may be used include the SV40 early promoter (Bernoist and Chambon, 1981, Nature 290: 304-310), and the promoter
25 contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., 1980, Cell 22: 787-797), among others.

A variety of host vector systems may be utilized to express
30 the peptide encoding sequence. These include mammalian cell systems infected with virus for example, vaccinia virus and adenovirus; insect cell systems infected with virus, for example, baculovirus; microorganisms for example yeast containing yeast vectors; or bacterial transformed with

bacteriophage, DNA, plasmid DNA, or cosmid DNA.

When a peptide of the invention has been recombinantly expressed, it may be isolated and purified by standard
5 methods including chromatography for example, ion exchange, affinity, and sizing column chromatography, centrifugation, differential solubility, or by any other standard technique for the purification of proteins and peptides.

10 As indicated above, an alternative to administration of a peptide of the invention is the administration of a nucleic acid molecule encoding the peptide, that is to say, using gene therapy.

15 Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

For general reviews of the methods of gene therapy, see
20 Goldspiel et al., 1993, Clinical Pharmacy 12:488-505; Wu and Wu, 1991, Biotherapy 3.:87-95; Tolstoshev, 1993, 30 Ann. Rev. Pharmacol. Toxicol. 32:573-596; Mulligan, 1993, Science 260:926-932; and Morgan and Anderson, 1993, Ann. Rev. Biochem. 62:191-217; May, 1993, TIBTECH 11(5):155-215.

25 Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), 1993, Current Protocols in Molecular Biology, John Wiley & Sons, NY; and Kriegler, 1990, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY.

30

In a preferred aspect, the nucleic acid encoding a peptide of the invention for use in gene therapy is part of an expression vector that expresses peptide in a suitable host. In particular, such a nucleic acid has a promoter operably

linked to the peptide coding region, said promoter being inducible or constitutive, homologous or heterologous and, optionally, tissue-specific. In another particular embodiment, a nucleic acid molecule is used in which peptide
5 coding sequence and any other desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the peptide encoding nucleic acid, as described (Koller and Smithies, 1989, Proc. Natl. Acad. Sci. USA
10 86:8932-8935; Zijlstra et al., 1989, Naturc 342:435-438). In another embodiment, a nucleic acid encoding a peptide of the invention, preferably operably linked to a promoter, is delivered by gene therapy methods.

15 Delivery of the nucleic acid into a patient may be either direct, in which case the patient is directly exposed to the nucleic acid or nucleic acid-carrying vector, or indirect, in which case, cells are first transformed with the nucleic acid *in vitro*, then transplanted into the patient. These two
20 approaches are known, respectively, as *in vivo* or *ex vivo* gene therapy.

Numerous techniques are known in the art for the introduction of foreign genes into cells (see e.g., Loeffler and Behr,
25 1993, Meth. Enzymol. 217:599-618; Cohen et al., 1993, Meth. Enzymol. 217:618-644; Cline, 1985, Pharmac. Ther. 29:69-92).

In such an embodiment of the invention in which recombinant cells are used in gene therapy, a nucleic acid encoding a
30 peptide of the invention is introduced into the cells such that they are expressible by the cells or their progeny, for example, as described above, and the recombinant cells are then administered *in vivo* for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any

stem and/or progenitor cells which can be isolated and maintained *in vitro* can potentially be used, such as hematopoietic stem cells (HSC), stem cells of epithelial tissues such as the skin and the lining of the gut, embryonic heart muscle cells, liver stem cells (PCT Publication WO 94/08598, dated April 28, 1994), neural stem cells (Stemple and Anderson, 1992, Cell 71:973-985), or epithelial stem cells (ESCs) (Rheinwald, 1980, Meth. Cell Bio. 21A:229; Pittelkow and Scott, 1986, Mayo Clinic Proc. 61:771).

10

Various delivery systems are known and can be used to administer a nucleic acid that encodes a peptide of the invention, for example, encapsulation in liposomes (Langer, Science 249:1527-1533 (1990); Treat et al., in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353-365 (1989)), microparticles, microcapsules, recombinant cells capable of expressing the nucleic acid, receptor-mediated endocytosis (see, e.g., Wu and Wu, 1987, J. Biol. Chem. 262:4429-4432), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of introduction of a nucleic acid include intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The nucleic acid may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings for example, oral mucosa, rectal and intestinal mucosa, and may be administered together with other biologically active agents. Administration can be systemic or local.

30

The peptides of the present invention appear to stimulate axon regeneration and stimulate cell survival. The terms "stimulate" and "promote" are used interchangeably herein. The ability of a peptide that comprises the tetrameric

structural unit defined above to stimulate axon regeneration or to stimulate cell survival may be determined readily by any appropriate test, many suitable techniques being known. Examples of suitable tests are given in the Examples.

5

Briefly, the ability of a peptide of the invention to stimulate axon regeneration may be determined by an in vitro neurite outgrowth assay. In such an assay typically a co-culture assay, a feeder layer, for example, of fibroblasts is established, neurones are plated on the feeder layer and are cultured in the presence and absence of the compound under investigation. Any suitable neurones may be used, for example, cerebellar granule cell neurons, hippocampal, dorsal root ganglion, olfactory, motor, or dopaminergic neurones. Neurite length is then measured, generally by microscopy after staining, and thus the effect of the compound under investigation on neurite outgrowth and hence axon regeneration is determined. The neurons may be of any appropriate origin. A growth factor known to stimulate neurite outgrowth, for example, FGF2 or NGF may be used as a control.

The ability of a peptide of the invention to stimulate cell survival may be assessed by determining the effect of the peptide on cells maintained under conditions under which they die or unless provided with a supplement. For example, many types of cell will die if maintained in a serum-free growth medium unless the medium is supplemented with an appropriate agent that stimulates their survival, for example, a growth factor. The nature of the agent may differ with cell type. For example, FGF stimulates the survival of fibroblasts in a serum-free medium. FGF is also effective in stimulating neurone survival.

Accordingly, in one suitable assay the effect of a peptide of the present invention on cell survival may be assessed by maintaining cells in a medium in which they will die unless the medium is supplemented with an agent that stimulates
5 their survival, and determining the effect on cell survival of supplementation of the medium with the peptide. A growth factor known to stimulate survival of that type of cell may be used as a control, for example, FGF or insulin may be used for fibroblasts and for neurones.

10

Cell survival may be assessed by a biochemical assay, for example, by determining cell respiration, for example, by using the mitochondrial substrate MTS, which is [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-
15 sulphophenyl)-2H-tetrazolium, inner salt) and measuring optical density at 490 nm. Alternatively or in addition, cell survival may be assessed by determining the number of viable cells, or may be assessed morphologically, for example, after fixing the cells and, if desired, staining to
20 visualise the cytoskeleton, for example, with TRITC-conjugated falloidin.

As mentioned above, the peptides of the present invention appear to lack the mitogenic and apoptotic properties shown
25 by many conventional growth factors. This is a clear advantage as the risk of tumorigenesis and of apoptosis mitigates against the therapeutic use of growth factors.

A peptide may be tested for mitogenic activity by any
30 appropriate assay. Many suitable assays are well known. Increase in the number of cells cultured in the presence of an agent compared with the number cultured in its absence is a simple test. A widely used test for mitogenesis involves the use of bromodeoxyuridine (BrDU), which is incorporated into

the nucleus of cells entering the S phase of the cell cycle and hence is indication of the stimulation of mitosis. Cells are cultured in the presence of BrDU and in the presence and absence of a substance under investigation. The cells are
5 then stained using BrDU. The presence of BrDU staining indicates stimulation of mitosis and hence that the agent under investigation is mitogenic. A known mitogen, for example, FGF2, may be used as a control.

10 The following non-limiting Examples illustrate the invention.

EXAMPLES

EXAMPLE 1

15 General methods for the production of peptides

Peptides are prepared by solid phase synthesis using standard Fmoc chemistry. This is based on sequential addition of amino acid residues, the α -amino and side chain amino groups of which are protected by a Fmoc group, to the amino group of
20 a linker attached to an insoluble resin support. After removal of the protecting group from the amino acid at the end of the growing chain using piperidine, the next protected amino acid is added, giving a peptide attached through its carboxy terminus to the resin via a linker. This procedure is
25 repeated until the final product has been assembled. The product is then removed from the resin using trifluoroacetic acid.

To produce peptides having a C-terminal amide group,
30 methylbenzhdrylamine is used and for peptides having a free (unblocked) carboxy terminus traditional Merrifield resins are used. Acetylation of the N-terminus for blocked peptides is performed by reacting the peptide resin with a solution of acetic anhydride in dichloromethane in the presence of di-

isopropylethylamine after removal of the N- α -t-butoxycarbonyl by acidolysis using trifluoroacetic acid.

Peptide dendrimers (multiple antigenic peptides, MAP) are prepared by standard procedures. For example, lysine having its α -amino and side chain amino groups protected with an F-moc group is coupled to a standard resin via a standard linking group as above. The protecting groups are removed by piperidine and further similarly protected lysines are added, one to the α -amino group and one to the side chain amino group. This results in a triple lysine backbone with four F-moc protecting groups, that is to say, a four branch MAP core. If desired, another F-moc protected lysine can be added to each of the four amino groups of the previous product, giving an eight branch core.

To a four branch core prepared as described above, the amino acids of the desired peptide are added sequentially from the C-terminus, see Figure 5.

Cyclic peptides may be produced by synthesising linear peptides synthesised as described above with a cysteine residue flanking each end of the peptide sequence. The cysteine is coupled first to the resin followed by the other amino acids and finally by a cysteine at the N-terminus. The product is then cyclised by reacting the two side chain thiol groups with a 10% solution of iodine in methanol to form a disulphide bridge.

The peptides used in the Examples below were manufactured by Mimotopes (UK) Limited.

Example 2

Production of peptide A(d)

Peptide A(d) has the structure $[\{Ac-DRVEPYSSSTA\}_2-K]_2-K-OH$, that is to say, four units of the acetylated peptide DRVEPYSSSTA [SEQ.ID.NO.:14] are linked to a branched lysine tetramer backbone structure. The branched lysine backbone structure is prepared as described in Example 1 above. Peptide A(d) is also referred to herein as Ac-DRVEPYSSSTA[lys]2[lys].

The F-moc protective groups are removed from the four lysine amino groups of the backbone structure, then alanine is added followed by threonine, serine, serine, tyrosine, proline, glutamic acid, valine, arginine, and aspartic acid.

Example 3

15 Production of cyclic peptide

The linear peptides DRVEPYSSSTA [SEQ.ID.NO.:14] is synthesised as described above may be prepared but with a cysteine residues flanking each end of the peptide sequence, giving the peptide CDRVEPYSSSTAC [SEQ.ID.NO.:18]. The cysteine is coupled first to the resin followed by the other amino acids and finally by a cysteine at the N-terminus. The linear peptide is then cyclised by reacting the two side chain thiol groups with a 10% solution of iodine in methanol to form a disulphide bridge.

25

EXAMPLE 4

Axon regeneration

The ability of an exogenous factor to stimulate neurite outgrowth (axon regeneration) can be tested in a neurite outgrowth assay in vitro. The assay of choice should so simulate the in vivo conditions that the results are indicative of potential in vivo activity of the agents tested. The stimulating activity of a test agent can be

compared with that of known neurite outgrowth-stimulating agents such as NGF and FGF2.

An example of such an assay is a co-culture assay in which
5 neurons are cultured on top of cellular monolayers for 16 hours, before being fixed and stained with a neuron-specific antibody. The mean length of the longest neurite per cell can then be determined by fluorescence microscopy using an appropriate image analysis programme. The neurons may be,
10 for example, (in this case cerebellar granule cell neurons, hippocampal, dorsal root ganglion, olfactory, motor or dopaminergic neurons. The cellular monolayer is, for example NIH 3T3 fibroblasts. .

15 The effect of various peptides of the invention on axon regeneration was determined using the following in vitro neurite outgrowth assay.

General Method

20 Monolayers of NIH 3T3 fibroblasts were established by seeding 80,000 cells per well in Lab-Tek 8-chamber slides previously coated sequentially with poly-L-lysine and fibronectin and culturing them overnight in DMEM+10%FCS growth medium.

25 Cerebellum was dissected from post-natal day 2-4 rat pups and the meninges and extraneous tissue discarded. Cerebellum was chopped into small pieces and trypsinised for 10 minutes at 37°C. Trypsin was neutralised using growth medium and the cells pelleted at 1000 x g for 7 minutes. Cerebellar neurons
30 were then resuspended in SATO growth medium containing 2%FCS, counted, and plated on top of the fibroblast monolayer at a density of 1,500 neurons per well. After 16 hours, cocultures were fixed with 4% para-formaldehyde and stained with an anti-GAP43 neuron-specific antibody, which was a gift from Dr

Graham Wilkin, Imperial College Department of Biochemistry, London, but any other neuron-specific marker can be used.

The mean neurite length of 150-200 neurons per well was
5 determined using a fluorescent microscope and Zeiss KS300 imaging software.

Example 4a

Method

10 Peptides A, A(d), B, C, D, E, and F were each incorporated in the SATO medium at a range of concentrations from 0 to 200 $\mu\text{g/ml}$. Nerve growth factor (NGF) and fibroblast growth factor (FGF2) were used as controls at the same range of concentrations.

15

Results

All of the peptides tested stimulate an 80-120% increase in neurite length (axon regeneration) of the rat cerebellar granule neurons cultured on monolayers of fibroblasts (NIB
20 3T3 cells). This response at least equals, and usually surpasses, the response of those neurons to nerve growth factor (NGF) and fibroblast growth factor (FGF) in the same assay.

25 In this neurite outgrowth assay, the peptide concentrations giving maximal effects are:

| | $\mu\text{g/ml}$ | μM |
|-----------------|------------------|---------------|
| Peptide A | 100-200 | 100-200 |
| 30 Peptide A(d) | 1.5-2.5 | ~0.4 |
| Peptide B | 75 | 150 |
| Peptide C | 38 | 75 |
| Peptide D | 63 | 100 |
| Peptide E | 50-100 | 50-100 |

Peptide F 100 100

Example 4b

In the neurite outgrowth assay described in the General
5 Method above, the mean neurite length approximately doubles
over the 16 hour culture period when a neurite outgrowth-
stimulating factor is included in the co-culture medium.
Such factors include NGF, FGF2, soluble NCAM.

10 When a synthetic 13 amino acid peptide, Ac-SIDRVEPYSSSTAQ-
amide, derived from the first fibronectin type III domain of
NCAM, was included in the coculture medium, a dose-dependent
increase in neurite outgrowth was seen, until at 100 μ M the
neurite length equalled that stimulated by the known neurite
15 outgrowth-stimulating molecule NCAM (Fig 1a). The full
activity was retained in the 10 amino acid peptide Ac-
DRVEPYSSSTA-amide (Fig 1b), and even the 4 amino acid peptide
Ac-DRVE-amide could stimulate the response (Fig 1c). The
graphs show that at low concentrations the peptides *inhibit*
20 neurite outgrowth stimulated by NCAM, but at higher
concentrations, they are themselves able to mimic NCAM-
stimulated neurite outgrowth. A peptide with the sequence
Ac-PYSSTA-amide was able to inhibit NCAM-stimulated neurite
outgrowth, but was itself unable to stimulate neurite
25 outgrowth (Fig. 1d).

By introducing amino-acid substitutions into the 'parent'
DRVEPYSSSTA sequence, the importance of various amino acids
can be seen. Thus Ac-DRSEPYSSSTA-amide and Ac-DAVEPYSSSTA-
30 amide retain the full ability to stimulate neurite outgrowth,
while Ac-ARVEPYSSSTA-amide and Ac-ARSEPYSSSTA-amide do not see
Fig 1e. However, it was predicted that multimeric versions
of even those peptides which do not stimulate neurite
outgrowth as monomers would have neurite outgrowth-

stimulating activity, since the evidence, see below, suggests that these peptides bind and activate FGF receptors.

A peptide Ac-EGME-amide stimulates neurite outgrowth to the same extent as Ac-DRVE-amide (Fig 1 g,h). A peptide EGMEGM is equally active. A multimeric peptide, Ac-DRVEPYSSSTA[lys]₂[lys]-OH, consisting of four copies of the peptide linked to a triple-lysine backbone ie [{Ac-DRVEPYSSSTA}₂ -K]₂ -K-OH, Peptide A(d), was found to stimulate neurite outgrowth with 250 times more potency than the monomeric peptide, stimulating maximal neurite outgrowth at 400 nM and with a bi-phasic dose-response curve (Fig 1f).

All the evidence shows that these peptides are binding to and activating FGF receptors to stimulate neurite outgrowth. FGF2 stimulates neurite outgrowth in this assay with a biphasic dose-response curve (Fig 1i). Low concentrations of the monomeric 10-mer Ac-DRVEPYSSSTA-amide (25µM) inhibit neurite outgrowth stimulated by FGF2 (Fig 1j). Cerebellar neurones taken from transgenic mice expressing a dominant-negative form of FGF receptors such that they are unable to signal through the FGF receptor are unable to extend neurites in response the monomeric 10-mer peptide Ac-DRVEPYSSSTA-amide (Fig 1k). This shows that FGF receptors are required for the neurite outgrowth effect of the peptide Ac-DRVEPYSSSTA-amide and demonstrates that the peptides of the invention bind to and activate FGF receptors.

Example 5

30 Promotion of cell survival

The ability of an agent to stimulate cell survival in vivo, i.e. to prevent cells from undergoing premature apoptosis, can be predicted by its ability to stimulate cell survival in vitro in a defined cell survival assay. A suitable assay is one in which cells are plated at low density in serum-free

medium for a number of days, one, two or three, for example, during which time they die unless the medium is supplemented with a survival factor. In such an assay, the activity of the test agent can be compared with that of known survival factors such as insulin.

Example 5a: Survival of fibroblasts

The ability of Peptide A(d) to stimulate survival of 3T3 fibroblasts was demonstrated in the following assay.

10

Method

NIH 3T3 fibroblasts were plated in each well of a 96-well microtitre plate at a density of 7,000 cells per well in DMEM containing 3 % FCS and left to attach overnight. The following day the growth medium was removed and replaced with 0.1 ml DMEM supplemented with FGF or Peptide A(d) or both, eight wells for each condition. The negative control was DMEM alone (maximal cell death) and the positive control was supplementation with 0.5 % FCS (maximal cell survival).

20

Six days later (seven days from first plating the cells), the relative number of living cells in each well was assessed by adding the mitochondrial substrate MTS, [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium, inner salt), for four hours before reading the optical density on a microtitre plate reader at a wavelength of 490 nm. The optical density gives a measure of the number of respiring cells present in each well at the end of the assay period.

30

In addition to the biochemical assay, survival was assessed qualitatively using a morphologic method in which cells were plated in 8-chamber Lab Tek slides in DMEM alone (negative control), or DMEM supplemented with 0.5 ng/ml FGF2; 0.5% FCS (positive control), or 25-200 µg/ml Peptide A(d). After three

35

days the cells were fixed with 4% paraformaldehyde, permeabilised with methanol and stained with TRITC-conjugated falloidin to visualise the cell cytoskeleton.

5 Results

When 3T3 fibroblasts are maintained at low density in serum-free DMEM, they die unless the medium is supplemented with an agent stimulating their survival. FGF is such a survival factor for 3T3 fibroblasts. When Peptide A(d) was added to the fibroblast culture medium alone at 25 $\mu\text{g/ml}$ it stimulated the survival of 3T3 fibroblasts to the same extent as 0.125 ng/ml FGF2. When DMEM was supplemented with Peptide A(d) together with FGF2, cell survival was greater than with either agent alone.

15

Discussion

The effects of Peptide A(d) on cell survival were very striking. All concentrations were at least as good as the positive control, 0.5 % FCS, at stimulating survival. In this experiment, there were far fewer cells remaining in the presence of FGF than in the presence of the peptide.

Example 5b: Survival of neurons

Using the same survival assays as those described above Peptide A(d) has been shown to stimulate survival of cerebellar neurons.

Example 6

Stimulation of cell survival

30 Method

NIH 3T3 cells are plated in 96-well plates at a density of 5,500 cells/well in 100 μl DMEM containing 0.5% FCS. After 24 hours, this medium is aspirated from the wells and replaced with 100 μl serum-free DMEM alone, or supplemented

with 0.5 % FCS (positive control), or a single potential survival factor, with four identical wells set up for each condition. The cells are left for up to seven days at 37 degrees and 8% CO₂ before the cell survival is determined using an MTS assay. In this assay 20 μ l of MTS substrate is added to each 100 μ l well and the plate left in the incubator for up to four hours. Respiring cells produce a coloured reaction product that can be read using a spectrophotometer at 490 nm. The coloured reaction product and hence the absorbance at 490 nm increases in proportion to the number of living (respiring) cells in the well. The mean absorbance at 490 nm \pm SEM is determined for the four identically set up wells. Wells containing a known survival factor such as insulin will contain more living cells after three days than cells cultured in unsupplemented medium.

Results

In the survival assay described above, FGF2 (very low concentrations - see later) and insulin stimulate dose-dependent survival of NIH3T3 cells in serum-free conditions. The results obtained using FGF2 are shown in Figure 2a,iii and the results using insulin in Figure 2a,i.

The 4-branched peptide Ac-DRVEPYSSSTA[lys]₂[lys] i.e. [{Ac-DRVEPYSSSTA}₂ -K]₂ -K-OH, Peptide A(d), stimulates dose-dependent survival fully mimicking and indistinguishable from survival stimulated by insulin and FGF2, and is able to stimulate survival in serum-free conditions for at least seven days (maximum time tested) (Fig. 2a,ii).

A 'scrambled' version of this peptide, namely Ac-ADTRSVSEYP[lys]₂[lys] i.e. [{Ac-ADTRSVSEYP}₂ -K]₂ -K-OH, was ineffective, but a "mutated" peptide with D/V and V/S amino acid substitutions, namely Ac-ARSEPYSSSTA [lys]₂[lys] i.e.

[{Ac- ARSEPYSSSTA}₂ -K]₂ -K-OH stimulated cell survival, though not as potently as the parent peptide Ac-DRVEPYSSSTA[lys]₂[lys] (Fig. 2b). This shows that peptides which do not stimulate neurite outgrowth as monomers are
5 nonetheless able to stimulate cell survival and should also stimulate neurite outgrowth as multimers. Peptides such as PYSSTA therefore, which do not appear to stimulate neurite outgrowth themselves, and peptides containing the PYSSTA motif will also be able to stimulate neurite outgrowth and
10 cell survival as multimers.

The monomeric peptides DRVE and EGME in the form Ac-DRVE-amide and Ac-EGME-amide were able to stimulate cell survival (Fig 2c), in an effect equivalent to that of the dendrimeric
15 Peptide A(d). The evidence shows that all these peptides act by binding and activating FGF receptors, since the peptide and FGF are specifically unable to stimulate the survival of a cell line which lacks FGF receptors, the L6 cell line (Fig 2d). Figure 2d compares the effects of DMEM, FCS, FGF2 at
20 0.1, 1 and 10 ng/ml, insulin at 0.5 and 5 µg/ml, and Peptide A(d) at 100 and 400 µg/ml.

Although low concentrations of FGF2 stimulate survival of NIH3T3 cells, higher concentrations (1 ng/ml) are ineffective
25 and 10 ng/ml stimulates apoptosis in these low-density cultures, i.e. after three days culture in the presence of DMEM supplemented with 10 ng/ml FGF2 there are fewer cells remaining than in wells containing unsupplemented DMEM (Fig 2e,i). This apoptotic effect of FGF2 can be reversed by
30 further supplementing the 10 ng/ml FGF-containing wells with PD, an inhibitor of MAP kinase signalling (Fig. 2e,ii).

Further examples of stimulation of cell survival

A Western blot (Fig 2f) was obtained as follows: one million NIH 3T3 cells were serum starved for 24 hours then challenged with the following agents: DMEM, 10% FCS, 0.1 ng/ml FGF2, 1 ng/ml FGF2, 10 ng/ml FGF2, 5µg/ml insulin, 400 µg/ml peptide 5 52 for 10 minutes at 37 degrees Celcius before being lysed in sample buffer and band separated by PAGE. Proteins were transferred to PVDF membranes and probed with an antibody that recognises tyrosine phosphorylated MAP kinase (upper panel). Triggering with 10% FCS, and 1 or 10 ng/ml FGF2 10 resulted in MAP kinase activation, while insulin showed a small activation and 0.1 ng/ml FGF2 and 400 µg/ml peptide did not. The membrane was stripped of antibody and re-probed with a MAP kinase antibody which does not discriminate between phosphorylated and non-phosphorylated MAP kinase 15 (lower panel). This shows that the amount of MAP kinase was the same in all lanes.

The results shown in Figure 2f demonstrate that FCS, 1 and 10 ng/ml FGF2 stimulate MAP kinase activation, while 0.1 ng/ml 20 FGF2, peptide A(d) i.e. Ac-DRVEPYSSSTA[lys]2[lys], and insulin do not. Thus the peptide mimics the effects of low concentrations of FGF2, namely stimulation of neurite outgrowth and cell survival, but not the apoptotic effect of higher concentrations of FGF2.

25

The effect of Peptide A(d) on the survival of DRG neurons was demonstrated as follows. DRG neurons were taken from embryonic day 16 rates and cultured at a density of 10,000 cells/well of a 96-well plate over 10 days in SATO medium, 30 unsupplemented or supplemented with 10ng/ml NGF, 10 ng/ml FGF2 or 800 ng/ml Peptide A(d). An OD of 100% reflects the baseline cell survival n the absence of any supplement in the medium. Figure 2g shows that NGF at 10 ng/ml increases cell survival by 121%, while FGF2 at 10 ng/ml increases survival

by 73%, and the peptide A(d) at 800 ng/ml increases survival by 51%.

NIH 3T3 cells were cultured for three days in the presence of DMEM alone, or supplemented with insulin at 0.5 µg/ml, FGF2 at 0.1 and 1 ng/ml, or Peptide A(d) at 10 ng/ml. Cells were then fixed and actin filaments visualised using TRITC-conjugated phalloidin to show the 3T3 morphology.

Cells do not survive well in the absence of supplements i.e. in DMEM alone. In contrast, supplementation with insulin, low concentrations of FGF2 (0.1 ng/ml) or Peptide A(d) stimulate cell survival. The morphology of the cells in the presence of these agents is indistinguishable. In the presence of higher concentrations of FGF2 (1 and 10ng/ml), the cells are reduced in number, and appear apoptotic, with bright and rounded morphology. These results are shown in Figure 2i.

Example 7

Oligodendrocyte survival

Oligodendrocytes are the myelinating glia of the central nervous system. They can be cultured in vitro, but require the inclusion of certain survival factors in the growth medium in order to survive in vitro. One such survival factor is insulin. Purified cultures of oligodendrocyte precursors provide an in vitro assay system in which to test for agents which stimulate oligodendrocyte survival, and may be expected to be beneficial for stimulating oligodendrocyte survival in vivo in conditions of demyelination, e.g. multiple sclerosis.

Method

Oligodendrocyte progenitors (O4 positive) are purified from postnatal day 4 rat cerebral cortex. First a trypsinised preparation of cortical cells is dissociated to single cell suspension and pre-plated to remove unwanted adherent cells such as microglia and macrophages. Oligodendrocyte progenitors express the O4 antigen, and can be purified by panning on petri-dishes coated with anti O4 antibodies. Unbound cells are removed by gentle washing, then O4 positive cells are removed by triturating them off the plate with DMEM containing 1% FCS, through a flame-polished pasteur pipette. O4 positive cells are plated on polyornithine-coated glass coverslips at a density of 500,000 cells per well in minimal growth medium containing insulin or the peptide Ac-DRVEPYSSSTA[lys]2[lys] i.e. $[\{\text{Ac-DRVEPYSSSTA}\}_2\text{-K}]_2\text{-K-OH}$, Peptide A(d), for six days, before being fixed and stained with the anti O4 antibody. Viable O4 positive cells are counted for each condition

20

Results

Fig. 2h shows that after six days in culture in medium supplemented with insulin (positive control) 25% of the cells originally plated survive. When the medium is supplemented with the peptide A(d), a dose-dependent survival effect is seen, such that at 25 $\mu\text{g/ml}$ 22% of the cells survive. This is comparable to the survival seen in the presence of insulin, a known survival factor for these cells.

30 **Example 8**

Peptide A(d) does not stimulate mitogenesis

FGF stimulates axonal regeneration, cell survival and mitogenesis. Peptide A(d) stimulates axonal regeneration and cell survival to a comparable degree. However, when cells are

observed growing in the presence of FGF2 or Peptide A(d) it is clear that, unlike FGF2, Peptide A(d) does not stimulate mitogenesis. This can be seen by the failure of Peptide A(d)-treated cells to show the rounding-up characteristic of cell division, and there is not the increase in cell number observed in the presence of a mitogenic stimulation by FGF2.

The following assays are used to quantitate any mitogenic effect of the peptide:

10

1. Standard Mitogenic Assay: A quiescent monolayer of 3T3 fibroblasts in serum-free DMEM is treated with FGF or Peptide A(d) in the presence of bromodeoxyturidine (BrDU) for four hours. After this time, cells are fixed and then stained for bromodeoxyuridine which is incorporated only into the nucleus of cells entering S phase of the cell-cycle, an indication of stimulation of mitogenesis. If the same number of cells incorporate BrDU in DMEM alone or supplemented with Peptide A(d) then the peptide does not stimulate mitogenesis. In the presence of FGF there should be a large increase in the number of cells incorporating BrDU as FGF is an established mitogen.

2. Cell count: 3T3 cells growing in DMEM/0.5 % FCS are counted using a graticule before and after the medium is supplemented with Peptide A(d) or FGF2 for four days. Any increase in cell number observed in the presence of either agent will indicate a mitogenic effect.

30 **Example 9**

Mitogenesis

FGF2 is a mitogen for a range of cells, including NIH3T3 cells. When the cells are plated at low density (5,500 cells per well), 10 ng/ml FGF2 stimulates apoptosis. However, when

the cells are plated more densely (11,000 cells per well), 10 ng/ml FGF2 stimulates cell proliferation (Fig.3a), as does insulin. The peptide A(d) does not stimulate proliferation.

- 5 The mitogenicity of an agent can be determined by testing its ability to stimulate entry of cells into 'S' phase in readiness for cell division. This is done by standard methodology and involves incubating cells in the presence of a potential mitogen in DMEM supplemented with
- 10 bromodeoxyuridine. After six hours the cells are fixed and stained with an antibody recognising bromodeoxyuridine. Cells in S phase can be distinguished by their incorporation of bromodeoxyuridine into DNA.
- 15 In this assay, the mitogenicity of 10 ng/ml FGF2 can be seen, but the peptide A(d) again mimics low concentrations (0.1 ng/ml) FGF2 in being unable to stimulate entry into S phase (Fig 3b). Thus FGF2 is a mitogen at high concentrations, but the peptide is not mitogenic at any of the concentrations
- 20 tested. Thus as a trophic and neuritogenic therapeutic the peptide has advantages over FGF2 in terms of safety: use of FGF2 runs the risk of stimulating uncontrolled cell division and tumour formation.

CLAIMS

1. A peptide which consists of or comprises the tetrameric peptide structural unit:

5

Xaa-Xaa-Xaa-Xaa [SEQ.ID.NO.:1]

in which Xaa at position 1 represents Glu or Asp, Xaa at position 2 represents any amino acid, Xaa at position 3
10 represents any amino acid and Xaa at position 4 represents Glu or Asp, each of the meanings of Xaa being independent.

2. A peptide as claimed in claim 1, which has an acyl substituent at the N-terminus and/or an amide group at the
15 carboxy terminus.

3. A peptide as claimed in claims 1 or claim 2, which comprises two cysteine residues that form a disulphide bond, giving a cyclic peptide.

20

4. A peptide as claimed in any one of claims 1 to 3, wherein in SEQ.ID.NO.:1 the amino acids at positions 2 and 3 are selected from natural amino acids, unnatural amino acids, and modified amino acids.

25

5. A peptide as claimed in any one of claims 1 to 3, wherein the amino acids in positions 2 and 3 of SEQ.ID.NO.:1 are selected from arginine (R), glycine (G), methionine (M) and serine (S).

30

6. A peptide as claimed in any one of claims 1 to 3, wherein SEQ.ID.NO.:1 is DRVE [SEQ.ID.NO.:6], EGME [SEQ.ID.NO.:7], EMGE [SEQ.ID.NO.:8], DRSE [SEQ.ID.NO.:9],

DAVE [SEQ.ID.NO.:10], EVRD [SEQ.ID.NO.:11] or EGGE
[SEQ.ID.NO.:12].

7. A peptide as claimed in any one of claims 1 to 6, which
5 comprises the tetrameric structural unit of SEQ.ID.NO.:1 as
part of a longer peptide molecule.

8. A peptide as claimed in claim 7, wherein a longer
peptide has up to 30 amino acids residues, for example, up to
10 25 amino acids residues, for example, up to 20 amino acids
residues, for example, up to 15 amino acids residues, for
example, 15, 14, 13, 12 , 11, 10, 9, 8, 7, 6, or 5 amino
acids residues.

15 9. A peptide as claimed in any one of claims 1 to 8, which
is or comprises the tetrameric structural unit of
SEQ.ID.NO.:1 and the sequence PYSSTA [SEQ.ID.NO.: 13] or part
of that sequence.

20 10. A peptide as claimed in claim 9, which has or comprises
an amino acid sequence selected from DRVEPYSSTA
[SEQ.ID.NO.:14], EGMEGM [SEQ.ID.NO.:15], DRSEPYSSTA
[SEQ.ID.NO.:16] and DAVEPYSSTA [SEQ.ID.NO.:17], and the
corresponding sequences having a cysteine residue at each
25 terminus.

11. A peptide that (i) consists of the amino acid sequence
PYSSTA [SEQ.ID.NO.: 13], or (ii) consists of three or more,
especially four or five contiguous amino acids of the peptide
30 PYSSTA, or (iii) comprises peptide PYSSTA or three or more,
especially four or five contiguous amino acids of peptide
PYSSTA.

12. A peptide that comprises all or part of the first

fibronectin type III repeat of NCAM.

13. A peptide as claimed in claim 12, which consists of a part of the first fibronectin type III repeat of NCAM

5 amino acid sequence that comprises the amino acid sequence DRVE.

14. A peptide as claimed in claim 12, which consists of a part of the first fibronectin type III repeat of NCAM

10 amino acid sequence that comprises the amino acid sequence PYSSSTA.

15. A peptide as claimed in any one of claims 1 to 14, which has one or more modifications selected from an acyl group at
15 the N-terminus, an amide group at the carboxy terminus and side chain modifications.

16. A peptide as claimed in any one of claims 1 to 15, in multimeric form, wherein the peptide and one or more further
20 peptides as claimed in claims 1 to 15 are linked together via a backbone structure.

17. A peptide in multimeric form as claimed in claim 16, wherein the backbone structure comprises one or more lysine
25 residues.

18. A peptide as claimed in claim 1, having the following structure:

Peptide A: Acetyl- DRVEPYSSSTA -amide;

30 Peptide B: Acetyl- DRVE -amide

Peptide C: Acetyl- EGME -amide

Peptide D: Acetyl- EGMEGM -amide

Peptide E: Acetyl- DRSEPYSSSTA -amide

Peptide F: Acetyl- DAVEPYSSSTA -amide

19. A peptide in multimeric form as claimed in claim 15 or claim 16, having the following structure:

Peptide A(d): [{Ac- DRVEPYSSSTA }₂ -K]₂ -K-OH

5 where Ac represents an acyl group, for example a lower acyl group, for example, having from 1 to 4 carbon atoms.

20. A peptide in multimeric form as claimed in claim 15 or claim 16, in which the group acetyl-DRVEPYSSSTA-(X)_n , acetyl-
10 DRVE-(X)_n , acetyl-EGME-(X)_n - , acetyl-EMGE-(X)_n - , acetyl-DRSE-(X)_n - , acetyl-DAVE-(X)_n - , acetyl-EVRD-(X)_n - , acetyl-EGGE-(X)_n - , acetyl-EGMEGM-(X)_n - , acetyl-DRSEPYSSSTA-(X)_n - or acetyl-DAVEPYSSSTA-(X)_n - is linked to a backbone structure, X representing a linker group and n representing an integer
15 of one or more, for example, 1,2 or 3.

21. A peptide in multimeric form as claimed in claim 20, wherein the backbone structure comprises three lysine molecules.

20

22. A peptide in multimeric form as claimed in claim 20 or claim 21, wherein the linker group is GS (glycine-serine) or a multiple thereof.

25 23. A peptide as claimed in any one of claims 1 to 22, in which the amino acid sequence of the peptide or of each peptide is reversed.

24. A pharmaceutical composition which comprises a peptide
30 as claimed in any one of claims 1 to 23 in admixture with a pharmaceutically suitable carrier.

25. A pharmaceutical composition as claimed in claim 24, in

a form suitable for oral or intravenous administration, or for topical administration.

26. A pharmaceutical composition as claimed in claim 25, in
5 a form suitable for application to a wound.

27. A pharmaceutical composition as claimed in claim 24, in a form suitable to enable the peptide to cross the blood-brain barrier.

10

28. A method of stimulating neurite outgrowth in a mammalian subject, especially a human subject, comprising administering to a subject in need of such treatment an amount of a peptide as claimed in any one of claims 1 to 23
15 effective for neurite outgrowth promotion.

29. A method of stimulating cell survival in a mammalian subject, especially a human subject, comprising administering to a subject in need of such treatment an amount of a peptide
20 as claimed in any one of claims 1 to 23 effective for said cell survival promotion.

30. A method as claimed in claim 29, wherein the cells are selected from neurones, oligodendrocytes and fibroblasts.

25

31. A method of treatment of a neurodegenerative disease in a mammalian subject, especially a human subject, comprising administering to a subject in need of such treatment an amount of a peptide as claimed in any one of claims 1 to 23
30 effective for said treatment.

32. A method as claimed in claim 31, wherein the neurodegenerative disease is motor neurone disease, multiple

sclerosis, Alzheimer's disease, Parkinson's disease, PSP (progressive supranuclear palsy) or a prion disease.

33. A method of treatment of a peripheral neuropathy in a mammalian subject, especially a human subject, comprising administering to a subject in need of such treatment an amount of a peptide as claimed in any one of claims 1 to 23 effective for said treatment.

34. A method as claimed in claim 33, wherein the peripheral neuropathy is diabetic neuropathy or chemotherapy-induced neuropathy.

35. A method of treatment for stimulating or restoring nerve function after trauma or surgery in a mammalian subject, especially a human subject, comprising administering to a subject in need of such treatment an amount of a peptide of the invention effective for said treatment.

36. A method as claimed in claim 35, wherein the nerve function is local nerve function or is paralysis caused by spinal cord injuries.

37. A method of stimulating angiogenesis in cardiac muscle in a mammalian subject, especially a human subject, comprising administering to a subject in need of such treatment an amount of a peptide of the invention effective for said treatment.

38. A method of treatment of ischaemia in a mammalian subject, especially a human subject, for example, ischaemia caused by a stroke, comprising administering to a subject in need of such treatment an amount of a peptide as claimed in any one of claims 1 to 23 effective for said treatment.

39. A method of inhibiting or reducing angiogenesis in a tumour in a mammalian subject, especially a human subject, comprising administering to a subject in need of such
5 treatment an amount of a peptide as claimed in any one of claims 1 to 23 effective for said inhibition.

40. A peptide as claimed in any one of claims 1 to 23 for use as a medicament.

10

41. Use of a peptide as claimed in any one of claims 1 to 23 for the manufacture of a medicament for stimulating neurite outgrowth in a mammalian subject, especially a human subject.

15 42. Use of a peptide as claimed in any one of claims 1 to 23 for the manufacture of a medicament for stimulating cell survival in a mammalian subject, especially a human subject.

20 43. Use of a peptide as claimed in claim 42, wherein the cells are selected from neurones, oligodendrocytes and fibroblasts.

44. Use of a peptide as claimed in any one of claims 1 to 23 for the manufacture of a medicament for treating a
25 neurodegenerative disease in a mammalian subject, especially a human subject.

45. Use of a peptide as claimed in claim 44, wherein the neurodegenerative disease is motor neurone disease, multiple
30 sclerosis, Alzheimer's disease, Parkinson's disease, PSP (progressive supranuclear palsy) or a prion disease.

46. Use of a peptide as claimed in any one of claims 1 to 23 for the manufacture of a medicament for the treatment of a

peripheral neuropathy in a mammalian subject, especially a human subject.

47. Use of a peptide as claimed in claim 46, wherein the
5 peripheral neuropathy is diabetic neuropathy or chemotherapy-induced neuropathy.

48. Use of a peptide as claimed in any one of claims 1 to 23
for the manufacture of a medicament for stimulating or
10 restoring nerve function after trauma or surgery in a mammalian subject, especially a human subject.

49. Use of a peptide as claimed in claim 48, wherein the
nerve function is local nerve function or is paralysis caused
15 by spinal cord injuries.

50. Use of a peptide as claimed in any one of claims 1 to 23
for the manufacture of a medicament for stimulating
angiogenesis in cardiac muscle in a mammalian subject,
20 especially a human subject.

51. Use of a peptide as claimed in any one of claims 1 to 23
for the manufacture of a medicament the treatment of
ischaemia in a mammalian subject, especially a human subject,
25 for example, ischaemia caused by a stroke.

52. Use of a peptide as claimed in any one of claims 1 to 23
for the manufacture of a medicament for inhibiting or
reducing angiogenesis in a tumour in a mammalian subject,
30 especially a human subject.

53. A peptide as claimed in claim 40, wherein the medicament
is for use in a method as defined in any one of claims 28 to
39..

54. A nucleic acid that encodes a peptide having an amino acid sequence as defined in any one of claims 1, 3, 5 to 12 and 22.

5

55. A method as claimed in any one of claims 28 to 39, wherein there is administered to the subject an effective amount of a nucleic acid encoding a peptide as defined in any one of claims 1, 3, 5 to 12 and 22.

10

56. A method as claimed in claim 55, carried out using in vivo or ex vivo gene therapy.

57. Use of a nucleic acid encoding a peptide as claimed in any one of claims 1, 3, 5 to 12 and 22, for the manufacture of a medicament for a method of treatment as defined in any one of claims 41 to 52.

58. Use of a nucleic acid as claimed in claim 54 for the manufacture of a medicament for in vivo or ex vivo gene therapy.

59. A nucleic acid as claimed in claim 54 for use as a medicament.

25

60. A nucleic acid as claimed in claim 59, wherein the medicament is for in vivo or ex vivo gene therapy in a method of treatment as defined in any one of claims 41 to 52.

61. A host cell comprising a nucleic acid as claimed in claim 54 and elements necessary for the transcription and translation of the nucleic acid.

62. A method of producing a peptide as defined in any one of

claims 1, 3, 5 to 12 and 22, which comprises culturing a host cell as claimed in claim 56 under conditions such that the peptide is expressed.

5 63. A method for producing a peptide as claimed in any one of claims 1 to 23, which comprises chemically synthesising the peptide and, optionally, carrying out one of more of the following steps:

- (a) carrying out any desired modifications, and
- 10 (b) linking two or more peptides to a linker group or a backbone structure to form a peptide multimer.

64. A peptide as claimed in any one of claims 12 to 14, wherein the peptide is:

- 15 1) A peptide consisting of or comprising the first fibronectin type III repeat of NCAM;
- 2) A peptide consisting essentially of the first fibronectin type III repeat of NCAM or a peptide consisting essentially of or comprising a part of the first
- 20 fibronectin type III repeat of NCAM.
- 3) A peptide of 1) or 2) with other sequences from NCAM, for example one or more other NCAM domains.
- 4) A peptide of any one of 1), 2) or 3) as part of a fusion protein with non-NCAM sequences, e.g. an NCAM-Fc fusion
- 25 peptide.

65. A peptide as claimed in any one of claims 12 to 14, or claim 64 wherein the NCAM molecule is that of databank entry GI:3334473 (SEQ.ID.No. 21).

1/16

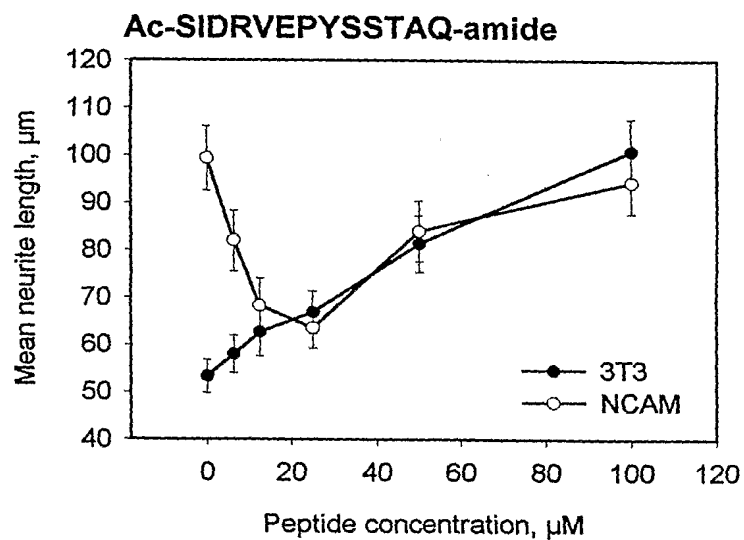


Figure 1a

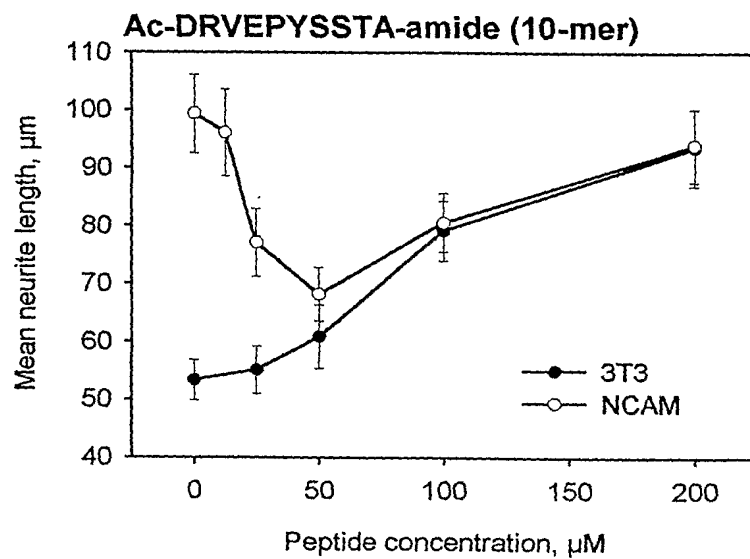


Figure 1b

2/16

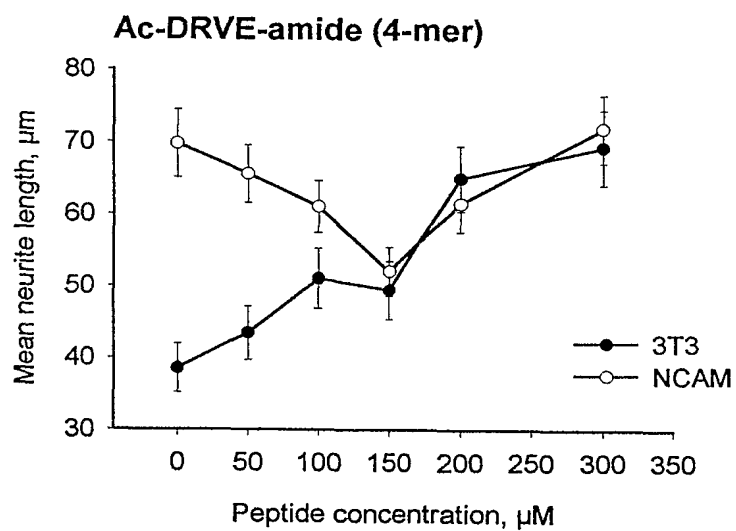


Figure 1c

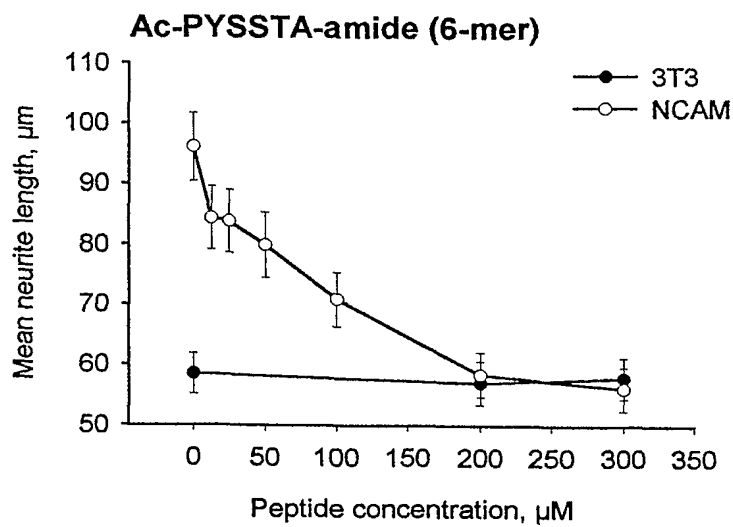


Figure 1d

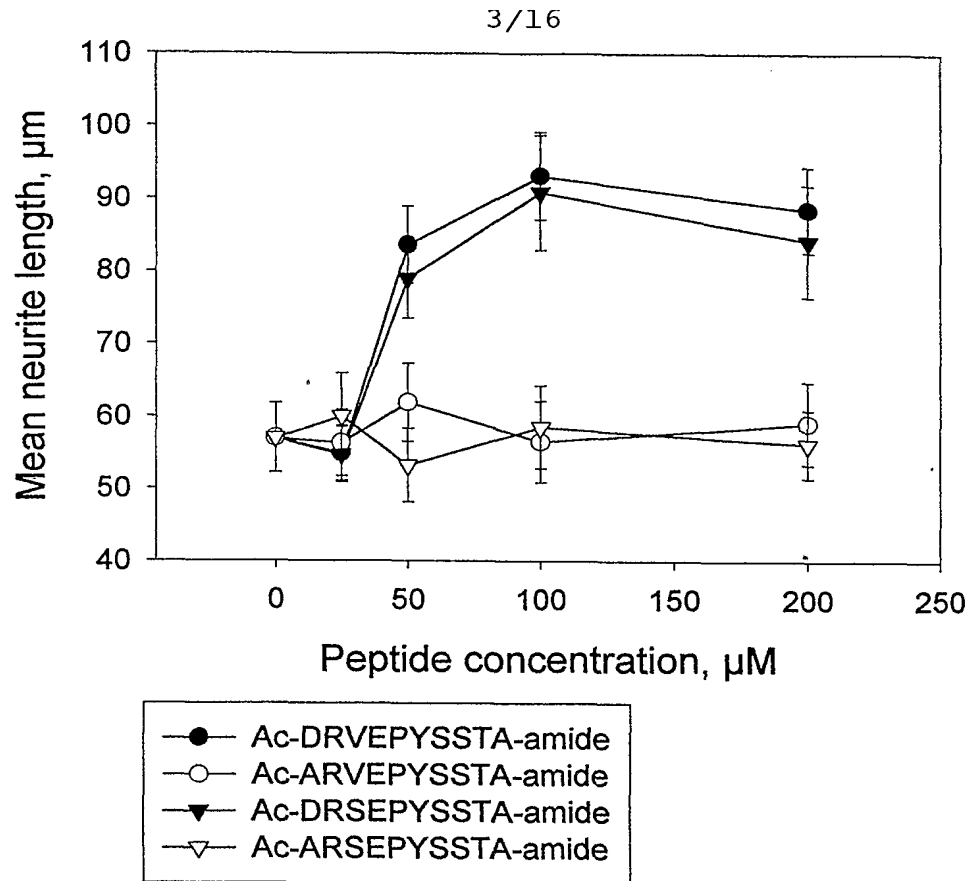


Figure 1e

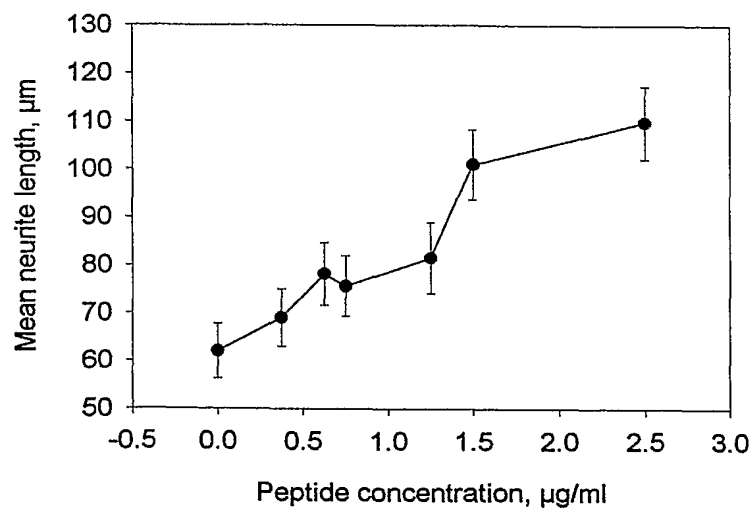


Figure 1f

4/16

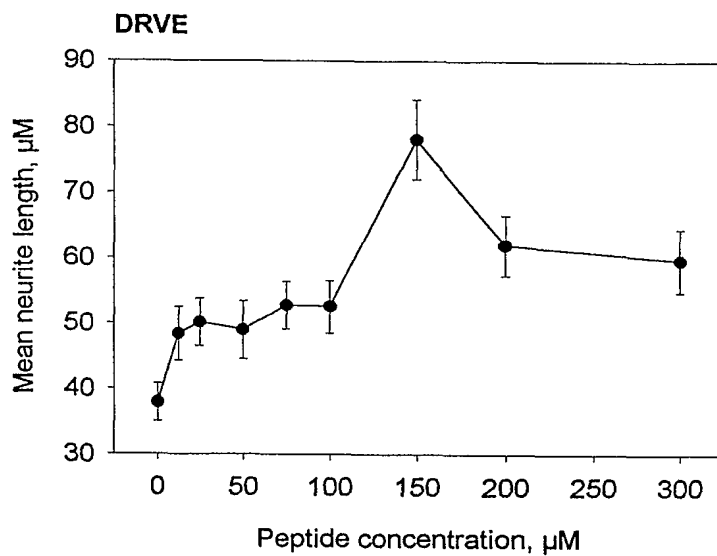


Figure 1g

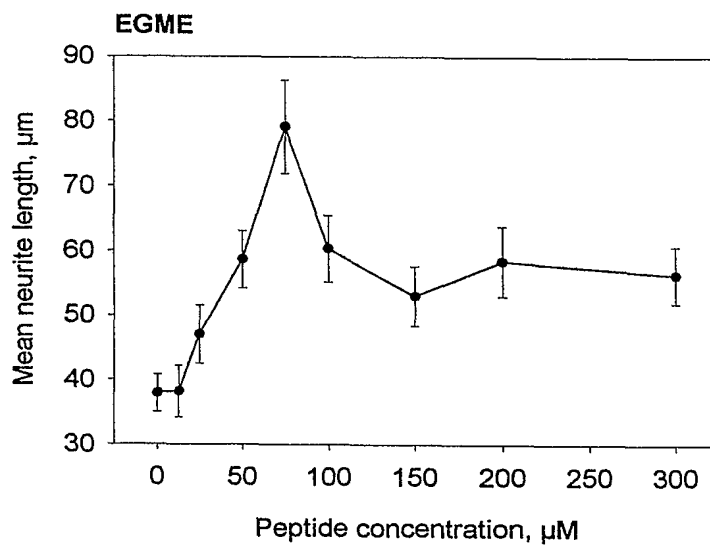


Figure 1h

5/16

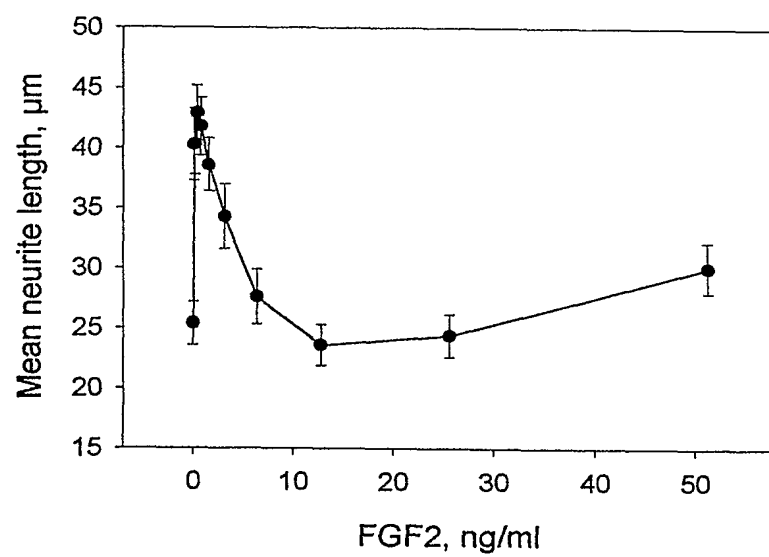


Figure 1i

6/16

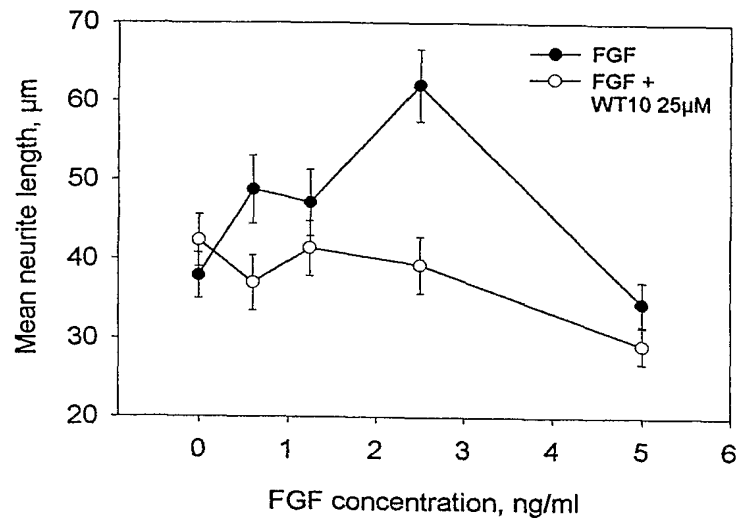


Figure 1j

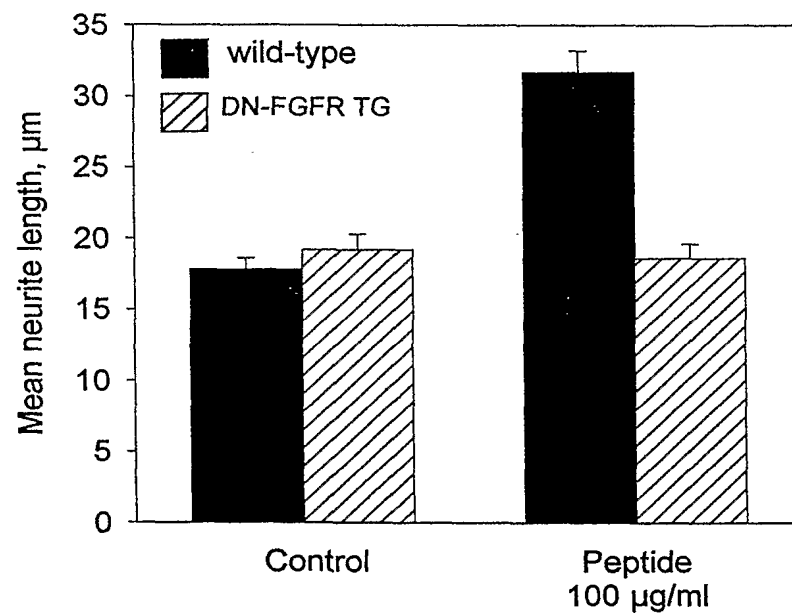


Figure 1k

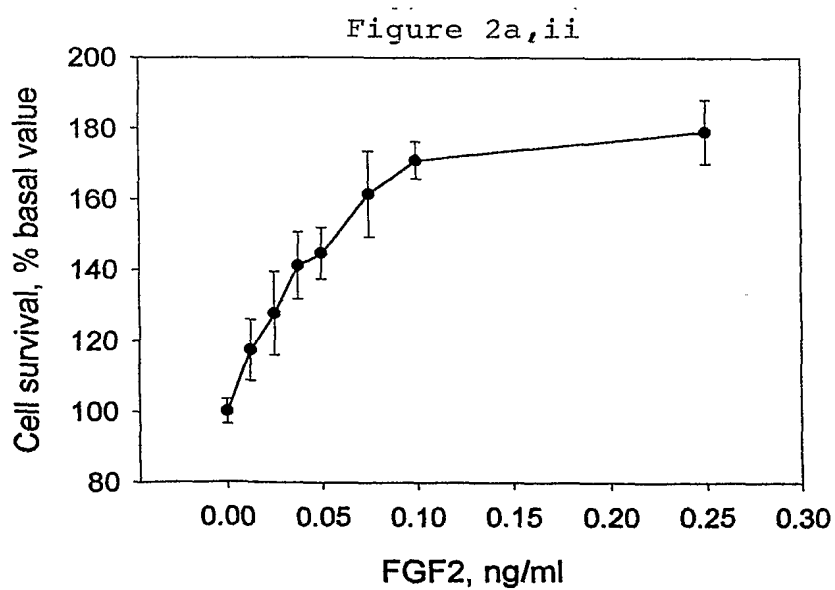
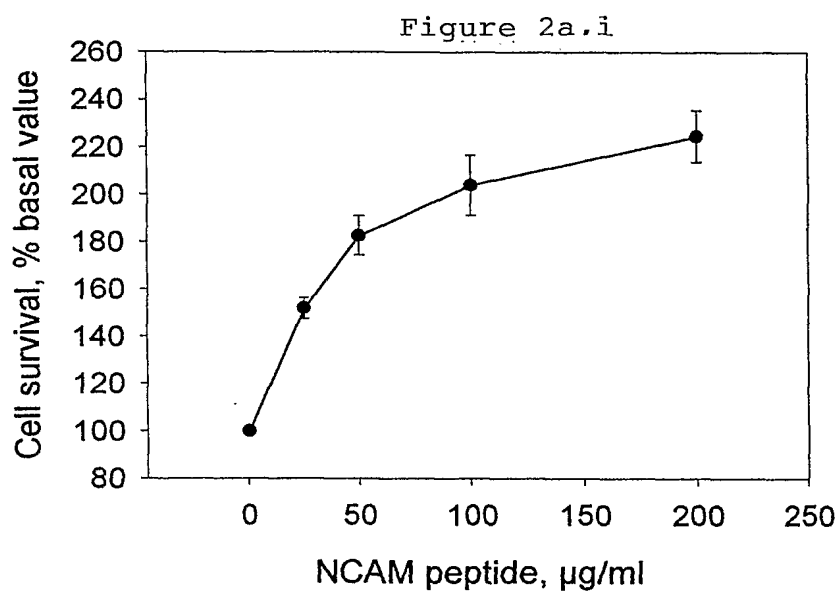
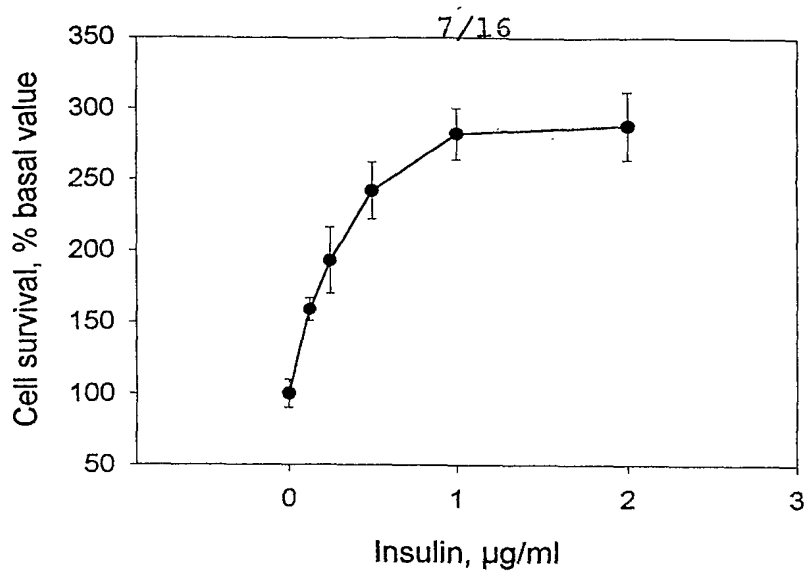


Figure 2a,iii

8/16

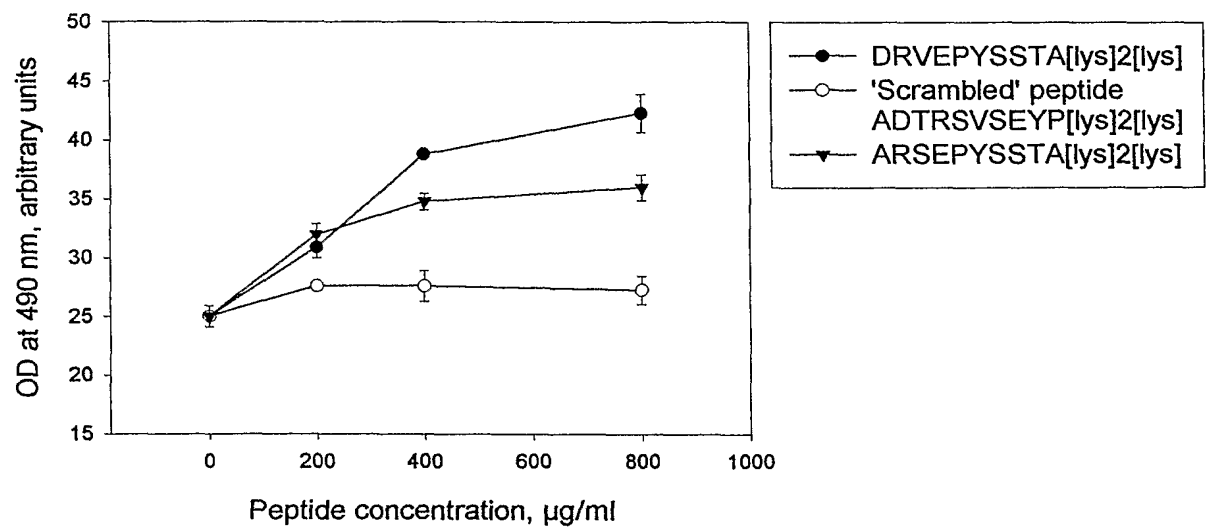


Figure 2b

9/16

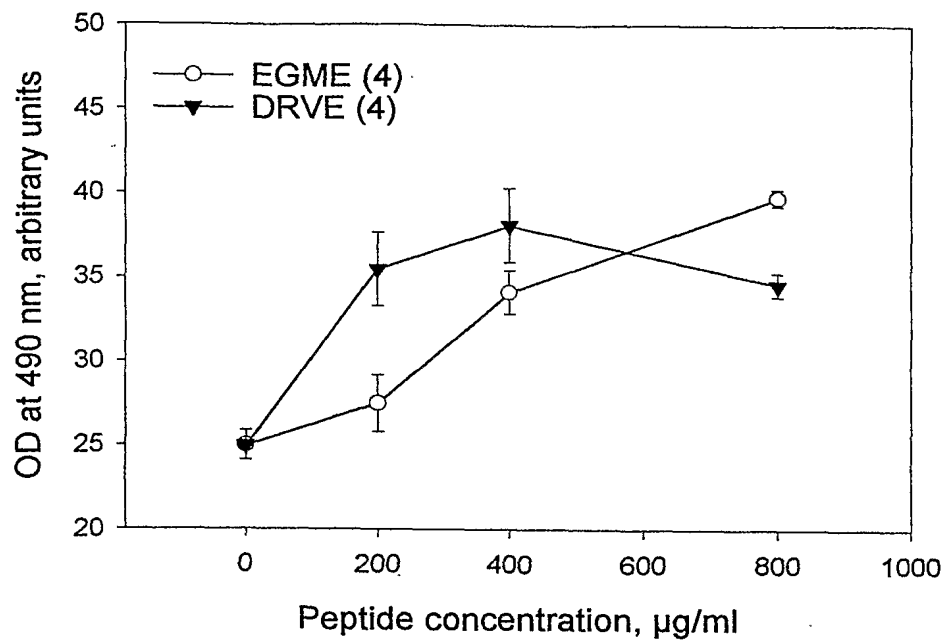


Figure 2c

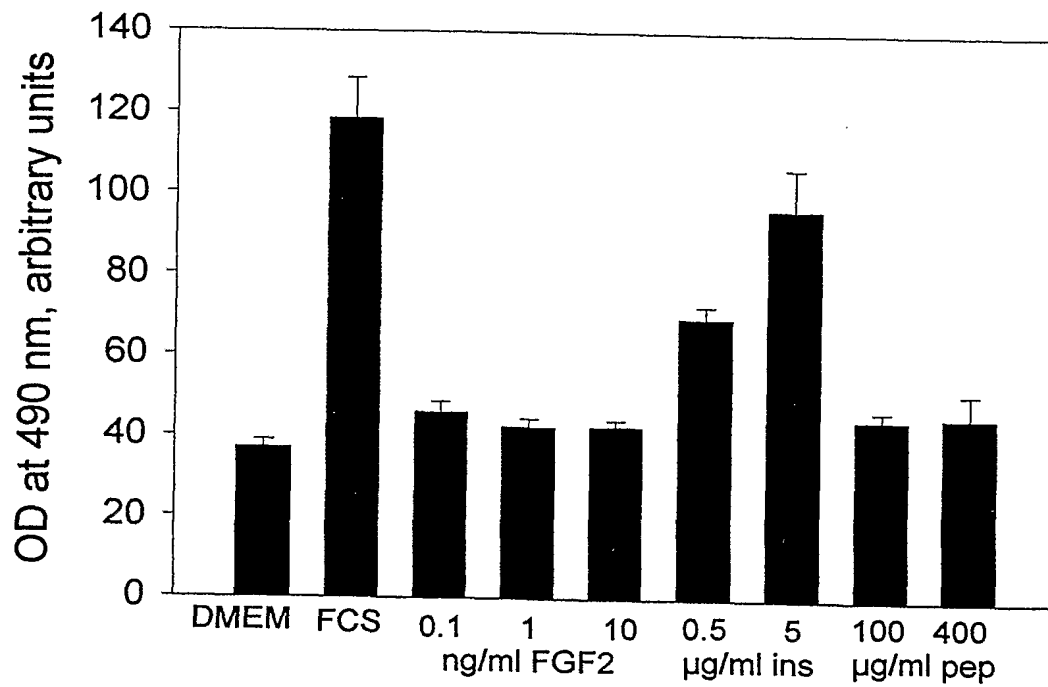


Figure 2d

10/16

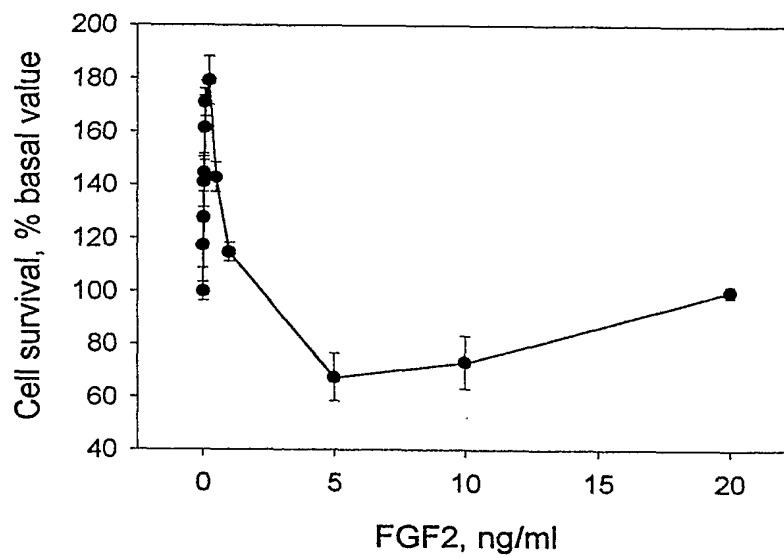


Figure 2e,i

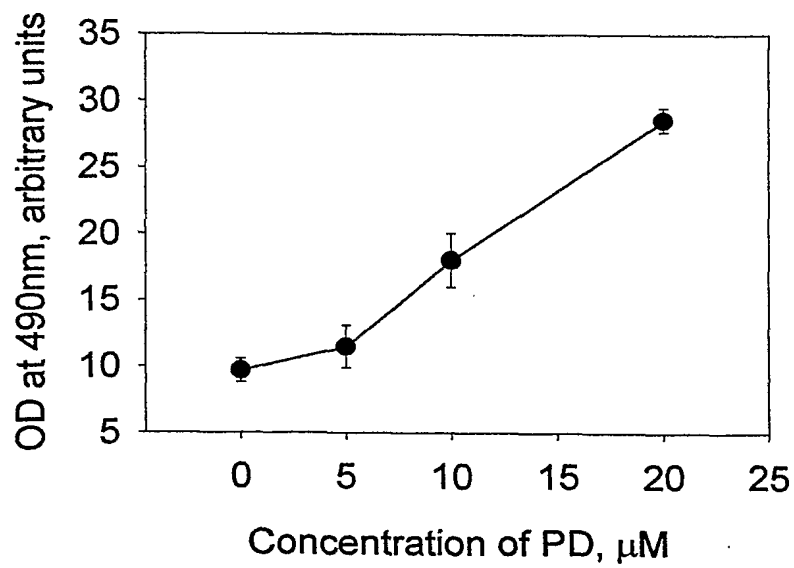


Figure 2e,ii

11/16

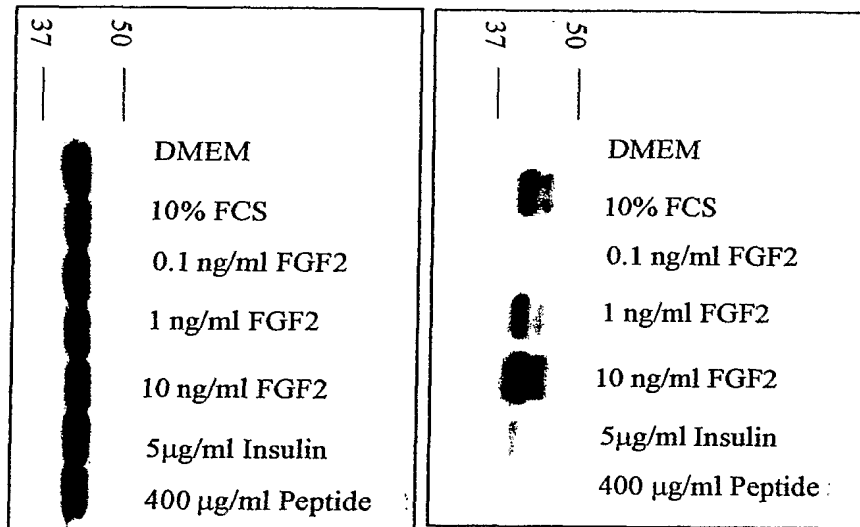


Figure 2f

12/16

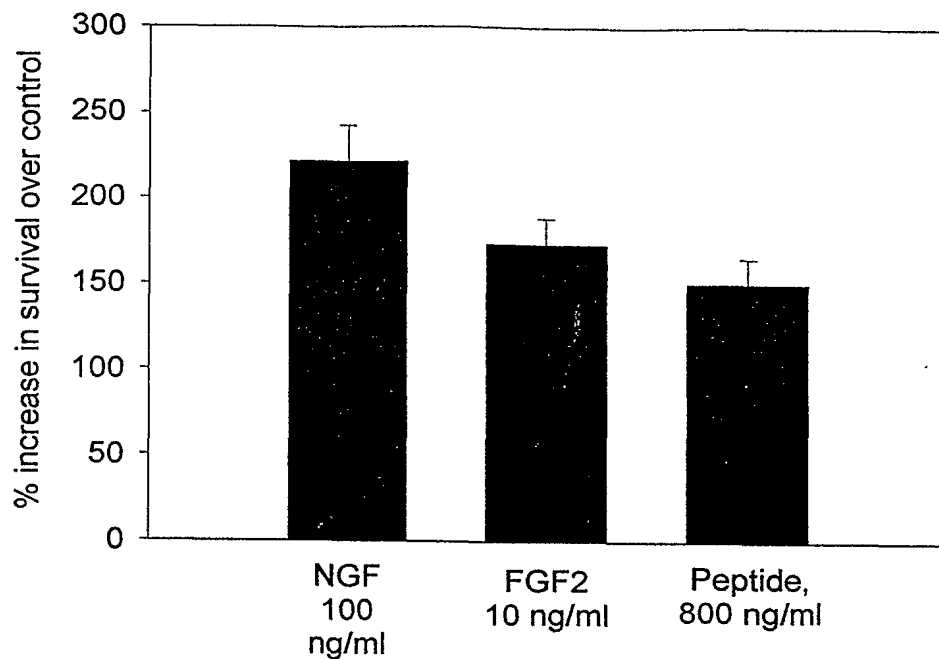


Figure 2g

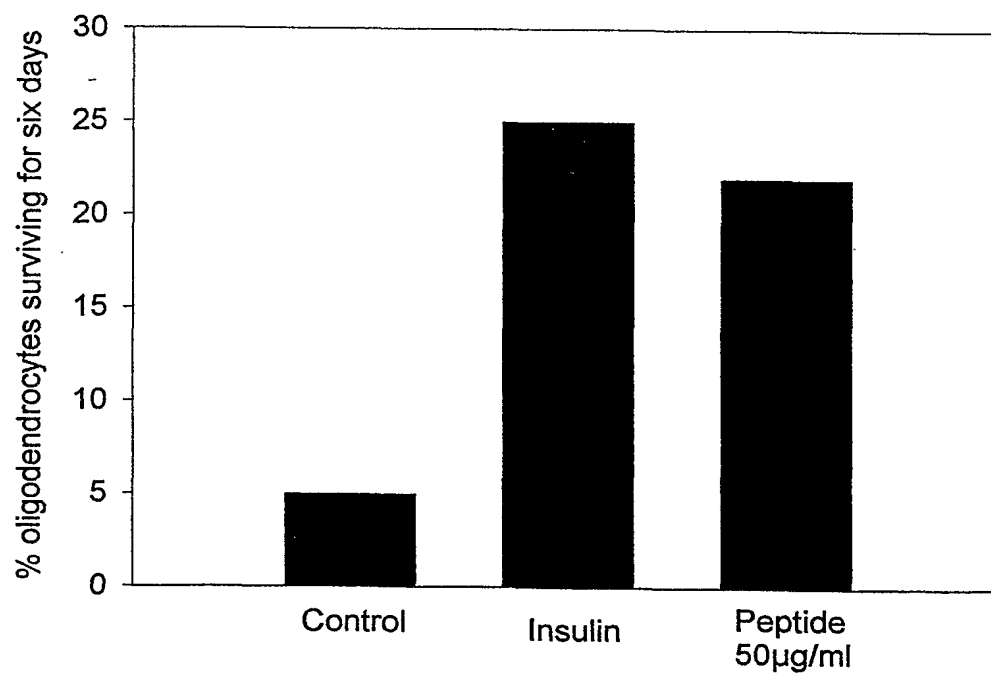


Figure 2h

13/16

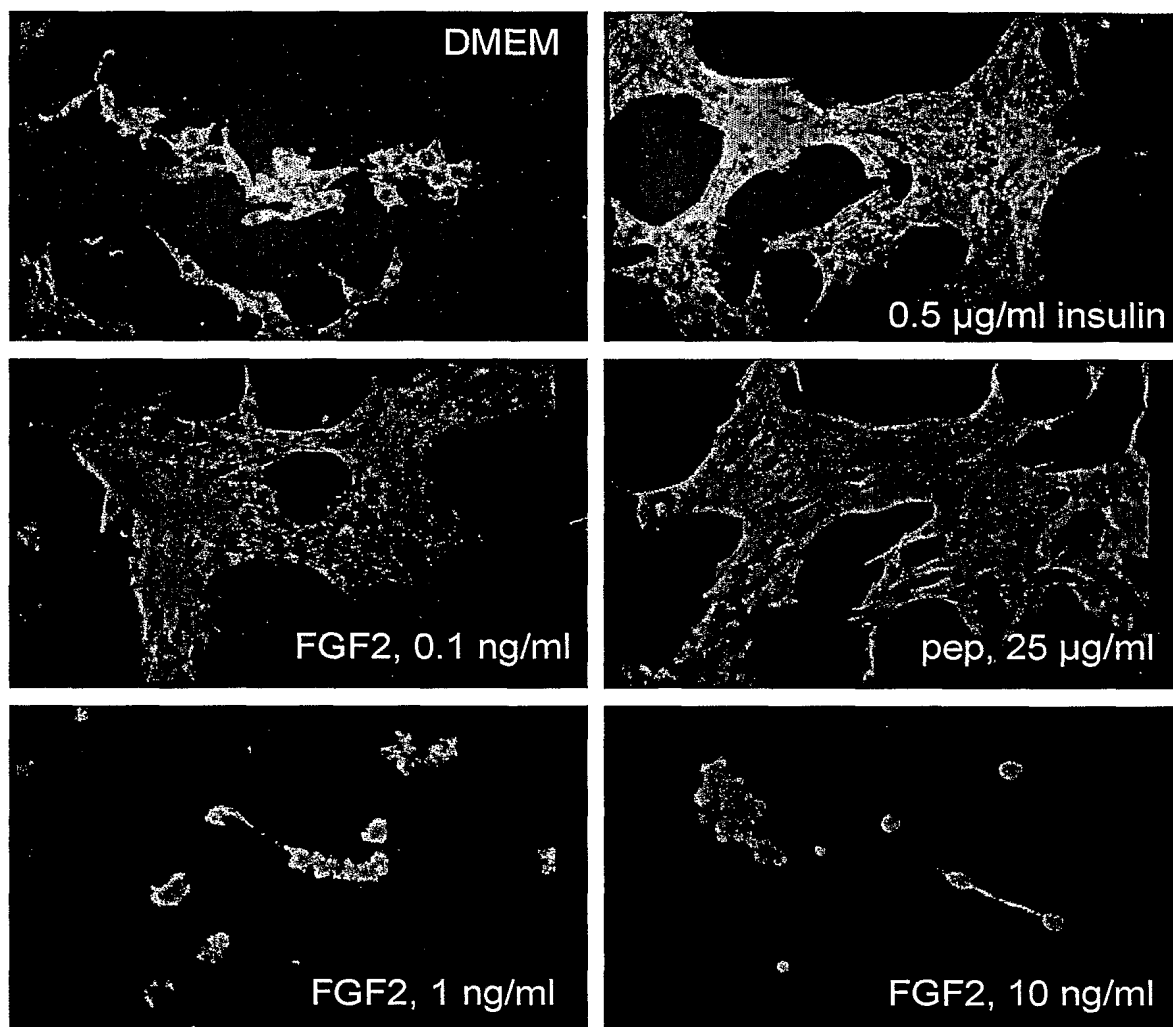


Figure 2i

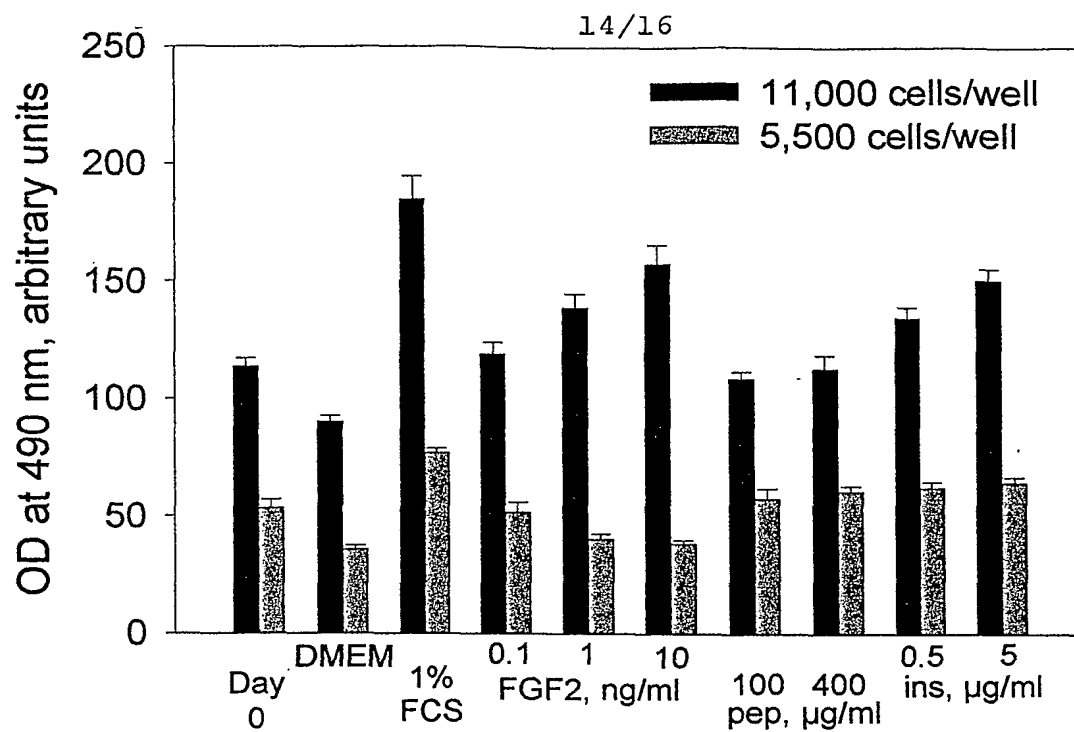


Figure 3a

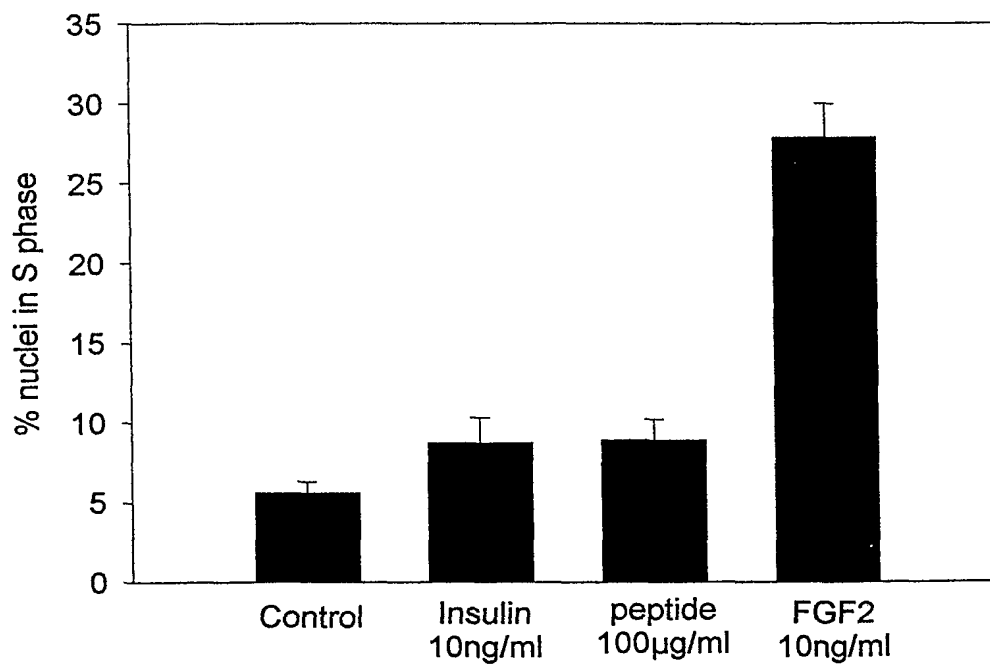


Figure 3b

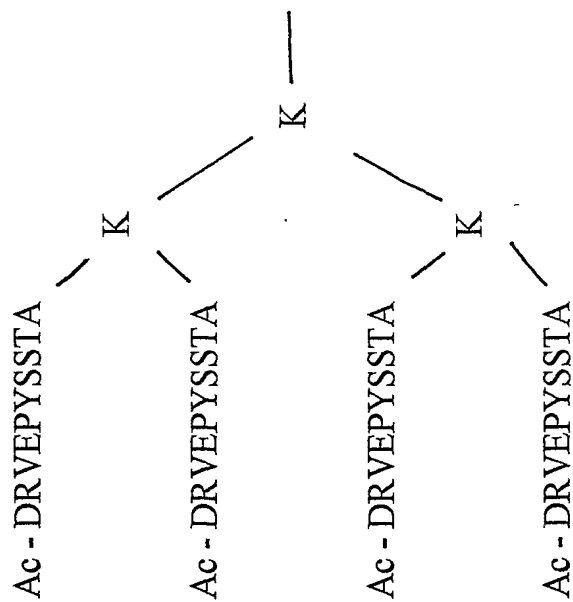


Figure 4

16/16

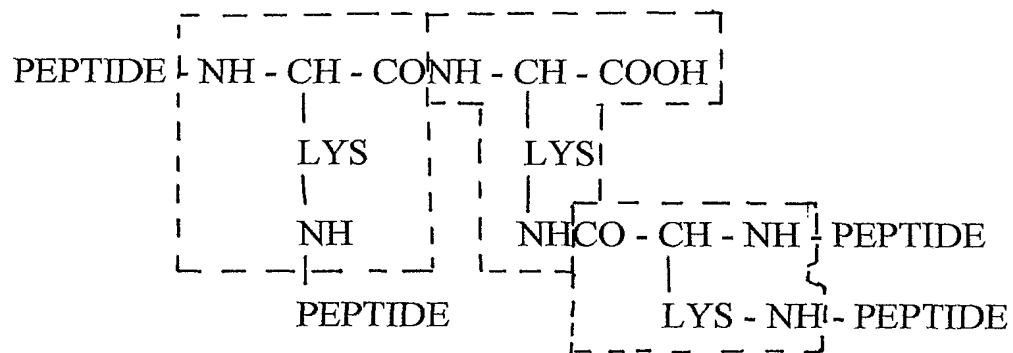


Figure 5

SEQUENCE LISTING

<110> Imperial College Innovations
Saffell, Jane

<120> PEPTIDES THAT PROMOTE CELL SURVIVAL AND AXON
REGENERATION

<130> 7245 JSvn TJH

<140>
<141>

<150> GB 0014870.0
<151> 2000-06-16

<160> 21

<170> PatentIn Ver. 2.1

<210> 1
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<221> PEPTIDE
<222> (1)..(4)
<223> Xaa at positions 1 & 4 is Glu or Asp, Xaa at
positions 2 & 3 is any amino acid

<220>
<223> Description of Artificial Sequence:PEPTIDE

<400> 1
Xaa Xaa Xaa Xaa
1

<210> 2
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<221> PEPTIDE
<222> (1)..(4)
<223> Xaa is at positions 2&3 is any amino acid

<220>

<223> Description of Artificial Sequence:PEPTIDE

<400> 2

Gln Xaa Xaa Gln

1

<210> 3

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1)..(4)

<223> Xaa at positions 2&3 is any animo acid

<220>

<223> Description of Artificial Sequence:PEPTIDE

<400> 3

Gln Xaa Xaa Asp

1

<210> 4

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1)..(4)

<223> Xaa at positions 2&3 is any amino acid

<220>

<223> Description of Artificial Sequence:PEPTIDE

<400> 4

Asp Xaa Xaa Gln

1

<210> 5

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1)..(4)

<223> Xaa at positions 2&3 is any amino acid

<220>

<223> Description of Artificial Sequence:PEPTIDE

<400> 5

Asp Xaa Xaa Asp

1

<210> 6

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1)..(4)

<220>

<223> Description of Artificial Sequence:PEPTIDE

<400> 6

Asp Arg Val Glu

1

<210> 7

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1)..(4)

<220>

<223> Description of Artificial Sequence:PEPTIDE

<400> 7

Glu Gly Met Glu

1

<210> 8
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<221> PEPTIDE
<222> (1)..(4)

<220>
<223> Description of Artificial Sequence:PEPTIDE

<400> 8
Glu Met Gly Glu
1

<210> 9
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<221> PEPTIDE
<222> (1)..(4)

<220>
<223> Description of Artificial Sequence:PEPTIDE

<400> 9
Asp Arg Ser Glu
1

<210> 10
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<221> PEPTIDE
<222> (1)..(4)

<220>
<223> Description of Artificial Sequence:PEPTIDE

<400> 10

Asp Ala Val Glu

1

<210> 11

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1)..(4)

<220>

<223> Description of Artificial Sequence:PEPTIDE

<400> 11

Glu Val Arg Asp

1

<210> 12

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1)..(4)

<220>

<223> Description of Artificial Sequence:PEPTIDE

<400> 12

Glu Gly Gly Glu

1

<210> 13

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1)..(6)

<220>

<223> Description of Artificial Sequence:PEPTIDE

<400> 13

Pro Tyr Ser Ser Thr Ala
1 5

<210> 14

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1)..(10)

<220>

<223> Description of Artificial Sequence:PEPTIDE

<400> 14

Asp Arg Val Glu Pro Tyr Ser Ser Thr Ala
1 5 10

<210> 15

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1)..(6)

<220>

<223> Description of Artificial Sequence:PEPTIDE

<400> 15

Glu Gly Met Glu Gly Met
1 5

<210> 16

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1)..(10)

<220>

<223> Description of Artificial Sequence:PEPTIDE

<400> 16

Asp Arg Ser Glu Pro Tyr Ser Ser Thr Ala
1 5 10

<210> 17

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1)..(10)

<220>

<223> Description of Artificial Sequence:PEPTIDE

<400> 17

Asp Ala Val Glu Pro Tyr Ser Ser Thr Ala
1 5 10

<210> 18

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1)..(12)

<220>

<223> Description of Artificial Sequence:PEPTIDE

<400> 18

Cys Asp Arg Val Glu Pro Tyr Ser Ser Thr Ala Cys
1 5 10

<210> 19

<211> 13

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:PEPTIDE

<220>

<221> PEPTIDE

<222> (1)..(13)

<400> 19

Ser Ile Asp Arg Val Glu Pro Tyr Ser Ser Thr Ala Gln

1

5

10

<210> 20

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:PEPTIDE

<220>

<221> PEPTIDE

<222> (1)..(10)

<400> 20

Ala Thr Ser Ser Tyr Pro Glu Val Arg Asp

1

5

10

<210> 21

<211> 848

<212> PRT

<213> Homo sapiens

<400> 21

Met Leu Gln Thr Lys Asp Leu Ile Trp Thr Leu Phe Phe Leu Gly Thr

1

5

10

15

Ala Val Ser Leu Gln Val Asp Ile Val Pro Ser Gln Gly Glu Ile Ser

20

25

30

Val Gly Glu Ser Lys Phe Phe Leu Cys Gln Val Ala Gly Asp Ala Lys

35

40

45

Asp Lys Asp Ile Ser Trp Phe Ser Pro Asn Gly Glu Lys Leu Thr Pro

50

55

60

| | | | | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asn | Gln | Gln | Arg | Ile | Ser | Val | Val | Trp | Asn | Asp | Asp | Ser | Ser | Ser | Thr | 65 | 70 | 75 | 80 |
| Leu | Thr | Ile | Tyr | Asn | Ala | Asn | Ile | Asp | Asp | Ala | Gly | Ile | Tyr | Lys | Cys | 85 | 90 | 95 | |
| Val | Val | Thr | Gly | Glu | Asp | Gly | Ser | Glu | Ser | Glu | Ala | Thr | Val | Asn | Val | 100 | 105 | 110 | |
| Lys | Ile | Phe | Gln | Lys | Leu | Met | Phe | Lys | Asn | Ala | Pro | Thr | Pro | Gln | Glu | 115 | 120 | 125 | |
| Phe | Arg | Glu | Gly | Glu | Asp | Ala | Val | Ile | Val | Cys | Asp | Val | Val | Ser | Ser | 130 | 135 | 140 | |
| Leu | Pro | Pro | Thr | Ile | Ile | Trp | Lys | His | Lys | Gly | Arg | Asp | Val | Ile | Leu | 145 | 150 | 155 | 160 |
| Lys | Lys | Asp | Val | Arg | Phe | Ile | Val | Leu | Ser | Asn | Asn | Tyr | Leu | Gln | Ile | 165 | 170 | 175 | |
| Arg | Gly | Ile | Lys | Lys | Thr | Asp | Glu | Gly | Thr | Tyr | Arg | Cys | Glu | Gly | Arg | 180 | 185 | 190 | |
| Ile | Leu | Ala | Arg | Gly | Glu | Ile | Asn | Phe | Lys | Asp | Ile | Gln | Val | Ile | Val | 195 | 200 | 205 | |
| Asn | Val | Pro | Pro | Thr | Ile | Gln | Ala | Arg | Gln | Asn | Ile | Val | Asn | Ala | Thr | 210 | 215 | 220 | |
| Ala | Asn | Leu | Gly | Gln | Ser | Val | Thr | Leu | Val | Cys | Asp | Ala | Glu | Gly | Phe | 225 | 230 | 235 | 240 |
| Pro | Glu | Pro | Thr | Met | Ser | Trp | Thr | Lys | Asp | Gly | Glu | Gln | Ile | Glu | Gln | 245 | 250 | 255 | |
| Glu | Glu | Asp | Asp | Glu | Lys | Tyr | Ile | Phe | Ser | Asp | Asp | Ser | Ser | Gln | Leu | 260 | 265 | 270 | |
| Thr | Ile | Lys | Lys | Val | Asp | Lys | Asn | Asp | Glu | Ala | Glu | Tyr | Ile | Cys | Ile | 275 | 280 | 285 | |
| Ala | Glu | Asn | Lys | Ala | Gly | Glu | Gln | Asp | Ala | Thr | Ile | His | Leu | Lys | Val | 290 | 295 | 300 | |
| Phe | Ala | Lys | Pro | Lys | Ile | Thr | Tyr | Val | Glu | Asn | Gln | Thr | Ala | Met | Glu | 305 | 310 | 315 | 320 |

| | | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|--|
| Leu | Glu | Glu | Gln | Val | Thr | Leu | Thr | Cys | Glu | Ala | Ser | Gly | Asp | Pro | Ile | | |
| | | | | 325 | | | | | 330 | | | | | 335 | | | |
| Pro | Ser | Ile | Thr | Trp | Arg | Thr | Ser | Thr | Arg | Asn | Ile | Ser | Ser | Glu | Glu | | |
| | | | 340 | | | | | 345 | | | | | 350 | | | | |
| Lys | Thr | Leu | Asp | Gly | His | Met | Val | Val | Arg | Ser | His | Ala | Arg | Val | Ser | | |
| | | 355 | | | | | 360 | | | | | 365 | | | | | |
| Ser | Leu | Thr | Leu | Lys | Ser | Ile | Gln | Tyr | Thr | Asp | Ala | Gly | Glu | Tyr | Ile | | |
| | 370 | | | | | 375 | | | | | 380 | | | | | | |
| Cys | Thr | Ala | Ser | Asn | Thr | Ile | Gly | Gln | Asp | Ser | Gln | Ser | Met | Tyr | Leu | | |
| 385 | | | | | 390 | | | | | 395 | | | | | 400 | | |
| Glu | Val | Gln | Tyr | Ala | Pro | Lys | Leu | Gln | Gly | Pro | Val | Ala | Val | Tyr | Thr | | |
| | | | | 405 | | | | | 410 | | | | | 415 | | | |
| Trp | Glu | Gly | Asn | Gln | Val | Asn | Ile | Thr | Cys | Glu | Val | Phe | Ala | Tyr | Pro | | |
| | | | 420 | | | | | 425 | | | | | 430 | | | | |
| Ser | Ala | Thr | Ile | Ser | Trp | Phe | Arg | Asp | Gly | Gln | Leu | Leu | Pro | Ser | Ser | | |
| | | 435 | | | | | 440 | | | | | 445 | | | | | |
| Asn | Tyr | Ser | Asn | Ile | Lys | Ile | Tyr | Asn | Thr | Pro | Ser | Ala | Ser | Tyr | Leu | | |
| | 450 | | | | | 455 | | | | | 460 | | | | | | |
| Glu | Val | Thr | Pro | Asp | Ser | Glu | Asn | Asp | Phe | Gly | Asn | Tyr | Asn | Cys | Thr | | |
| 465 | | | | | 470 | | | | | 475 | | | | | 480 | | |
| Ala | Val | Asn | Arg | Ile | Gly | Gln | Glu | Ser | Leu | Glu | Phe | Ile | Leu | Val | Gln | | |
| | | | | 485 | | | | | 490 | | | | | 495 | | | |
| Ala | Asp | Thr | Pro | Ser | Ser | Pro | Ser | Ile | Asp | Gln | Val | Glu | Pro | Tyr | Ser | | |
| | | | 500 | | | | | 505 | | | | | 510 | | | | |
| Ser | Thr | Ala | Gln | Val | Gln | Phe | Asp | Glu | Pro | Glu | Ala | Thr | Gly | Gly | Val | | |
| | | 515 | | | | | 520 | | | | | 525 | | | | | |
| Pro | Ile | Leu | Lys | Tyr | Lys | Ala | Glu | Trp | Arg | Ala | Val | Gly | Glu | Glu | Val | | |
| | 530 | | | | | 535 | | | | | 540 | | | | | | |
| Trp | His | Ser | Lys | Trp | Tyr | Asp | Ala | Lys | Glu | Ala | Ser | Met | Glu | Gly | Ile | | |
| 545 | | | | | 550 | | | | | 555 | | | | | 560 | | |
| Val | Thr | Ile | Val | Gly | Leu | Lys | Pro | Glu | Thr | Thr | Tyr | Ala | Val | Arg | Leu | | |
| | | | | 565 | | | | | 570 | | | | | 575 | | | |

| | | | | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala | Ala | Leu | Asn | Gly | Lys | Gly | Leu | Gly | Glu | Ile | Ser | Ala | Ala | Ser | Glu | 580 | 585 | 590 | |
| Phe | Lys | Thr | Gln | Pro | Val | Gln | Gly | Glu | Pro | Ser | Ala | Pro | Lys | Leu | Glu | 595 | 600 | 605 | |
| Gly | Gln | Met | Gly | Glu | Asp | Gly | Asn | Ser | Ile | Lys | Val | Asn | Leu | Ile | Lys | 610 | 615 | 620 | |
| Gln | Asp | Asp | Gly | Gly | Ser | Pro | Ile | Arg | His | Tyr | Leu | Val | Arg | Tyr | Arg | 625 | 630 | 635 | 640 |
| Ala | Leu | Ser | Ser | Glu | Trp | Lys | Pro | Glu | Ile | Arg | Leu | Pro | Ser | Gly | Ser | 645 | 650 | 655 | |
| Asp | His | Val | Met | Leu | Lys | Ser | Leu | Asp | Trp | Asn | Ala | Glu | Tyr | Glu | Val | 660 | 665 | 670 | |
| Tyr | Val | Val | Ala | Glu | Asn | Gln | Gln | Gly | Lys | Ser | Lys | Ala | Ala | His | Phe | 675 | 680 | 685 | |
| Val | Phe | Arg | Thr | Ser | Ala | Gln | Pro | Thr | Ala | Ile | Pro | Ala | Asn | Gly | Ser | 690 | 695 | 700 | |
| Pro | Thr | Ser | Gly | Leu | Ser | Thr | Gly | Ala | Ile | Val | Gly | Ile | Leu | Ile | Val | 705 | 710 | 715 | 720 |
| Ile | Phe | Val | Leu | Leu | Leu | Val | Val | Val | Asp | Ile | Thr | Cys | Tyr | Phe | Leu | 725 | 730 | 735 | |
| Asn | Lys | Cys | Gly | Leu | Phe | Met | Cys | Ile | Ala | Val | Asn | Leu | Cys | Gly | Lys | 740 | 745 | 750 | |
| Ala | Gly | Pro | Gly | Ala | Lys | Gly | Lys | Asp | Met | Glu | Glu | Gly | Lys | Ala | Ala | 755 | 760 | 765 | |
| Phe | Ser | Lys | Asp | Glu | Ser | Lys | Glu | Pro | Ile | Val | Glu | Val | Arg | Thr | Glu | 770 | 775 | 780 | |
| Glu | Glu | Arg | Thr | Pro | Asn | His | Asp | Gly | Gly | Lys | His | Thr | Glu | Pro | Asn | 785 | 790 | 795 | 800 |
| Glu | Thr | Thr | Pro | Leu | Thr | Glu | Pro | Glu | Lys | Gly | Pro | Val | Glu | Ala | Lys | 805 | 810 | 815 | |
| Pro | Glu | Cys | Gln | Glu | Thr | Glu | Thr | Lys | Pro | Ala | Pro | Ala | Glu | Val | Lys | 820 | 825 | 830 | |

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Thr | Val | Pro | Asn | Asp | Ala | Thr | Gln | Thr | Lys | Glu | Asn | Glu | Ser | Lys | Ala |
| | | 835 | | | | | 840 | | | | | 845 | | | |